INVESTIGATION OF COMPOUNDS OF HIGH CALORIC DENSITY

TECHNICAL DOCUMENTARY REPORT No. MRL-TDR-62-35

MAY 1962

BIOMEDICAL LABORATORY
6570th AEROSPACE MEDICAL RESEARCH LABORATORIES
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

Contract Monitor: Eugene G. Sander, 1/Lt, USAF
Project No. 7163, Task No. 716304

(Prepared under Contract No. AF 33(616)-6008
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1100 - August 1962 - 44-1953 & 1954

FOREWORD

The investigations described in this report are a continuation of the studies reported in WADD Technical Report 60-575 (August 1960). The research described in this report was carried out from 1 January 1960 to 30 December 1961 at the Department of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Cambridge 39, Massachusetts. The work was done under Contract AF 33(616)-6008, Project No. 7163, "Physiology Research," Task No. 716304, "Studies in Nutritional Physiology and Metabolism," for the Biospecialties Section, Physiology Branch, Biomedical Laboratory, 6570th Aerospace Medical Research Laboratories. Eugene G. Sander, 1/Lt, USAF, served as contract monitor. Personnel participating in the project were: Henry A. Dymsza, Ph.D., Samuel A. Goldblith, Ph.D., Sanford A. Miller, Ph.D., Steven R. Tannenbaum, Emily L. Wick, Ph.D. The authors are indebted to Dr. S. M. Nagy and his associates, Massachusetts Institute of Technology, for the microanalyses.

Animal experimentation was performed in accordance with the "Rules Regarding Animal Care" established by the American Medical Association.

ABSTRACT

Synthesis of 2, 4-dimethylheptanoic acid has been completed. Preliminary acute toxicity tests indicated that 2, 4-dimethylheptanoic acid has a low order of toxicity (LD_{50}-5 gm/kg) similar to other short-chain fatty acids. To facilitate metabolic studies, synthesis of the compound labeled C^{14} has begun and techniques for quantitative identification of probable metabolic products have been developed. Further studies were made of the factors influencing the caloric bio-assay. A series of animal metabolic studies has indicated that odd-carbon fatty acids may be partly glucogenic. In addition, 1, 3-butanediol was utilized for energy at approximately 6.0 cal/gm in high-fat diets. The slower growth of animals fed this compound at levels up to 20 percent of the diet was due to decreased food intake. Seven-month feeding tests have verified the effectiveness of 1, 3-butanediol and high-fat levels for dietary use under various conditions.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES (cont.)

LIST OF TABLES (cont.)

SECTION A. INTRODUCTION

In recent years, much scientific attention has been focused onto the problems associated with man's relationship to unusual environments, particularly those associated with travel in outer space. Of these relationships, the problem of meeting man's nutritional needs has occupied an increasingly important place. Certainly, there have not been adequate solutions to the problem of satisfying man's metabolic requirements under the conditions of confinement and stress associated with space flight. An extended space voyage would require that food be carried or produced during the flight, and this would have to be accomplished under conditions where storage space and load weight are severely limited. Obviously, therefore, all material that man requires for his continual existence, including food or food-producing ecological systems, need to be as concentrated, as light and as miniaturized as possible.

For short space flights, the conventional type of natural or dehydrated foods and feeding systems appear to be sufficient (ref. 1). In addition, nutritionally complete liquid diets could also be of value. However, for more extended operations during which some stored food may be desirable maximum reductions in weight and space are quickly attained with present processing techniques. Any additional reduction in space and weight requires a totally different concept of food and food materials.

A possible approach to this problem was introduced in WADD Technical Report 60-575 (ref. 2) entitled <u>High-Energy Metabolites</u>. Since satisfaction of the caloric need of man accounts for the largest portion of food consumed, studies were directed towards producing high energy diets by using "high-energy metabolites." High-energy metabolites were defined as known artificial or synthetic compounds which have a greater caloric density than the more usual sources of nutrients.

To review, WADD Technical Report 60-575 described the search for high-energy metabolites and methods of assaying for caloric density using a newly developed bio-assay and a direct-indirect animal calorimeter. Two promising compounds were developed in this research. The first, 1,3-butanediol, has a caloric density of approximately 6.0 Cal/gm and is possibly metabolized as a carbohydrate. The second, a methylated fatty acid of intermediate chain length, 2,4-dimethylheptanoic acid, may be oxidized in the body without producing ketosis. The synthesis of this compound was designed and completed.

The present report is concerned with the continuation of the previous studies.

Objectives

The objectives of this research were: (a) to study available compounds, other than the common aliments (proteins, fats, carbohydrates), for use along with, or admixed with, normal foodstuffs to provide metabolizable energy in the human or animal body; (b) to investigate the physiological effects and factors affecting the long-term feeding of high caloric density diets containing limited amounts of carbohydrate; (c) to synthesize and study new compounds which have high physiological fuel value and are efficiently utilized in the body, the caloric density of these compounds being of such high order that diets compounded with these compounds have a higher energy content than that normally attained with balanced feeding regimens fairly high in fat; and (d) to develop, improve, and use biological and physical (calorimetric) methods in the screening and evaluation of high-energy metabolites.

SECTION B. INVESTIGATION OF COMPOUNDS OF HIGH CALORIC DENSITY

I. Introduction

The goal of this study is to determine the main metabolic pathways for the utilization of 2,4-dimethylheptanoic acid (2,4-DMHA) in supplying energy to the animal body. This requires synthesis of the acid, study of its toxicity, synthesis of 2,4-dimethylheptanoic acid-2-methyl-C^{14}, and investigation of the metabolic end products derived from the labeled compound. Synthesis of the unlabeled compound and determination of its toxicity has been completed. Synthesis of the labeled acid by means of a malonic ester condensation is in progress. Since it is expected that the unoxidized portion of ingested 2,4-DMHA will consist of low molecular weight monocarboxylic and dicarboxylic acids, an analytical scheme has been developed for separation and identification of such compounds.

Reasons for interest in the utilizable energy value of 2,4-DMHA were discussed in WADD Technical Report 60-575 (ref. 2).

II. The Synthesis of 2,4-Dimethylheptanoic Acid

The synthesis of 2,4-DMHA is described below.

During the course of a routine check of its purity by gas chromatographic analysis of its methyl ester, it was noted that two components were present in comparable quantity. Since this observation indicated the possible presence of a hitherto undiscovered impurity in 2,4-DMHA, a detailed investigation was undertaken to disclose the nature of this impurity. Experimental evidence has shown that the two peaks in question represent the two diastereoisomers of 2,4-dimethylheptanoic acid, and further corroborate the theoretical structure of this compound.

Experimental

Apparatus

Gas chromatographic analyses were done on an instrument constructed in these laboratories employing a thermistor katharometer as detector. Two columns were used: (a) 1 meter, 4 mm i.d., 10% (w/w) Carbowax 4000 on 60 to 80 mesh diatomaceous earth and (b) 2 meter, 4 mm i.d., 10% (w/w) diethyleneglycol succinate (DEGS) on 60 to 65 mesh, acid-washed diatomaceous earth. Helium flow rate was 50 ml/min. Column temperatures were 50°C or 100°C.

Infrared spectra were obtained by means of a Beckman IR-5 spectrophotometer equipped with a KBr beam condenser.

Materials

2-Methylpentanol-1 (I) was dried and distilled twice before use, n_D^{20} 1.4185, b.p. 93-95°C (92 mm). Gas chromatographic analysis of I, after its purification, showed one peak on the Carbowax column at 100°C.

Diethyl methylmalonate (III) was distilled before use, n_D^{20} 1.4129, b.p. 194-196°C (760 mm). The purity of III was studied by its saponification, decarboxylation and esterification of the resulting free acids with diazomethane (ref. 3). Gas chromatographic analysis of the esters on the DEGS column at 50°C showed the presence of more than 99% methyl propionate and slightly less than 1% of methyl acetate. It was concluded that III had contained more than 99% diethyl methylmalonate and slightly less than 1% of diethyl malonate.

Diethyl malonate was more than 99% pure as determined by gas chromatography on the DEGS column at 100°C. A single, minor, unidentified peak was present.

2,4-Dimethylheptanoic acid (IV)

I was treated with PBr_3 according to the procedure of Noller and Dinsmore (ref. 4). 1-Bromo-2-methylpentane (II) was obtained in 55.6% yield, b.p. 42-43°C (15.5 mm), n_D^{20} 1.4480 (n_D^{20} 1.4495) (ref. 5). Gas chromatography of II on the Carbowax column at 100°C showed one peak. Its infrared spectrum contained no OH bands.

Anal. Calcd. for $C_6H_{13}Br$: C, 43.65; H, 7.94; Br, 48.44. Found: C, 43.47; H, 7.82; Br, 48.73.

Sodium t-butoxide (1.05 M, 24.15 gm sodium) in 800 ml of t-butyl alcohol was treated with III (1.10 M, 191.5 gm) followed by II (1.05 M, 173.2 gm). The resulting mixture was worked up in the conventional manner (ref. 6) to give the disubstituted malonic ester. The ester was saponified for 48 hours with 50% KOH. The free acid was decarboxylated by refluxing with constant boiling HCl for 24 hours. The free acid IV was obtained in 50% yield, b.p. 82-83°C (0.6 mm), n_D^{20} 1.4302.

Anal. Calcd. for $C_9H_{18}O_2$: C, 68.35; H, 11.39. Neutral equivalent 158.0. Found: C, 68.34; H, 11.15. Neutral equivalent 158.0 ± 0.5.

Esterification (ref. 3) of about 50 mg of IV yielded methyl 2,4-dimethylheptanoate (V).

4-Methylheptanoic acid (VIII) (ref. 7)

Sodium ethoxide (0.32 M, 7.36 gm sodium) in 150 ml of absolute ethanol, diethyl malonate (0.34 M, 54.5 gm) and 1-bromo-2-methylpentane (0.33 M, 54.5 gm) were reacted in the usual manner to give diethyl 2-methylpentylmalonate (VI), n_D^{20} 1.4287, b.p. 110-112°C (3.2 mm), in 73% yield. Gas chromatography of VI on the Carbowax column at 100°C showed one peak. Saponification of 5 gm of VI yielded 2-methylpentylmalonic acid (VII) from which was formed its bis-S-benzylthiouronium salt.

Anal. Calcd. for $C_{25}H_{36}N_4O_4S_2$: C, 57.66; H, 6.98; N, 10.78. Found: C, 58.16; H, 7.17; N, 10.42.

Decarboxylation of about 50 mg of VII and esterification of the resulting 4-methylheptanoic acid (VIII) yielded methyl 4-methylheptanoate, IX. Gas chromatography of IX on the DEGS column at 100°C showed one peak.

Separation of Diastereoisomers of V

Chromatography of methyl 2,4-dimethylheptanoate (V) on the DEGS* column at 100°C (flow rate 50 ml/min.) resulted in elution of two well separated peaks (A and B) within 10 min. The separation factor (α) (the ratio of the partition coefficients of peak A and peak B) was 1.09. Resolution (ref. 8) of the components was 1.07. The possibility that one of the components was methyl 4-methylheptanoate (VIII), methyl propionate, or 2,4-dimethylheptanoic acid was eliminated on the basis of the retention volumes of reference samples at the above conditions.

Peak A was collected as it was eluted from the DEGS column and re-chromatographed under the same conditions. Only one peak was obtained. The infrared spectrum of peak A, as a pure liquid and in carbon tetrachloride solution, was essentially identical to the spectrum of methyl 2,4-dimethylheptanoate (V). Normalization of the area under peaks A and B showed that peak A represented 53.2% of the total mixture.

Epimerization

1. Methyl 2,4-dimethylheptanoate was heated under reflux for 21 hours in the presence of 1 M sodium methoxide in anhydrous methanol. The infrared spectrum of the resulting ester, as a pure

* Although a number of liquid phases were investigated (Carbowax 4000, Carbowax 1500, Ucon LB-1715, Silicone SE-30, and Silicone QF-1), only DEGS effected a separation of the diastereoisomers of methyl 2,4-dimethylheptanoate.

liquid or in carbon tetrachloride was essentially identical with the spectrum of untreated methyl 2,4-dimethylheptanoate. Chromatography on the DEGS column showed the presence of peaks A and B. The area of peak A had decreased to 41.8% of the mixture.

2. An approximately 10% aqueous solution of sodium 2,4-dimethylheptanoate was heated 18 hours in a sealed tube at 115°C. The free acid was isolated and esterified with diazomethane. Separation of the ester on the DEGS column showed that peak A had increased to 56.2% of the total mixture of components A and B.

Discussion

The synthesis of 2,4-dimethylheptanoic acid was accomplished in a straightforward manner. The fact that its methyl ester was separated by gas chromatography into two almost equal components (A and B) suggested that diastereoisomers had been resolved. The possible presence of impurities, such as methyl 4-methylheptanoate, methyl propionate and free 2,4-dimethylheptanoic acid, was eliminated by comparison of the retention volumes of components A and B with those of the authentic reference compounds.

A and B were shown to be diastereoisomers by the fact that the infrared spectrum of A was essentially identical to the infrared spectrum of methyl 2,4-dimethylheptanoate, that the relative quantities of A and B were changed by epimerization, and that the infrared spectrum of the epimerized mixture was the same as that of the original methyl 2,4-dimethylheptanoate.

III. Toxicity Studies with 2,4-Dimethylheptanoic Acid

Initial studies of the acute toxicity of 2,4-DMHA and its sodium salt have been completed. These investigations attempted to estimate the LD_{50}(7 days) of these compounds and thus gain knowledge of the feasibility of their use as dietary components.

Objective

To determine, in the rat, the single lethal oral dose for 2,4-DMHA and its sodium salt.

Materials and Methods

Male Sprague-Dawley (Charles River Breeding Laboratory, Wilmington, Mass.) 200-gm rats were used. They were housed in individual, wire-bottomed cages and were fed water and Purina Lab Chow *ad libitum*. Food was removed from the cages 12 hours prior to intubation so that the animals' stomachs would be relatively empty.

2,4-DMHA was mixed in corn oil, while sodium 2,4-DMHA was mixed in water. Dose levels of 0.1 gm/kg to 10 gm/kg were intubated in a single 3-ml dose by means of a ball point intubation needle. Control animals were given the same volume of corn oil or water. After intubation, the animals were replaced in their cages, fed ad libitum and observed for 1 week.

Results and Discussion

The results of this study are summarized in Table 1. Based on these data, the LD_{50}(7 days) is estimated to be about 5 gm/kg, a level which places 2,4-DMHA in the "slightly toxic" category.

The toxic responses of the animals included: lack of coordination, polypnea, unconsciousness, narcosis, and death. Animals receiving the lowest dose levels showed, in general, none of these responses while those receiving intermediate levels demonstrated many of these symptoms. Death in the most severely stressed animals generally occurred within 24 hours after feeding.

This slight toxicity of 2,4-dimethylheptanoic acid and its sodium salt has been observed for almost all fatty acids of chain lengths less than 9 carbon atoms (ref. 9) and for triglycerides of these fatty acids (ref. 10). The toxicity is presumably due to the effect of low molecular weight fatty acid anions on the central nervous system. This effect, however, is not well understood.

It is expected, however, that this slight toxicity of 2,4-dimethylheptanoic acid will be reduced considerably in the rat when it is fed as part of a mixed diet, since dietary short-chain acids are commonly consumed without evidence of toxic responses. It is, therefore, expected that the observed toxicity of this compound will not be significant in terms of either the study of its in-vivo metabolism or its potential use as a high caloric density dietary supplement.

Summary

The oral LD_{50}(7 days) of 2,4-dimethylheptanoic acid and its sodium salt was estimated to be approximately 5 gm/kg. This slight toxicity is similar to those observed upon examination of other short-chain fatty acids and is not expected to be significant when this compound is fed as part of a mixed ration or when it is used for in-vivo metabolic studies.

IV. Synthesis of 2,4-Dimethylheptanoic Acid-2-Methyl-C^{14}

Preparation of labeled 2,4-DMHA required that its yield (based on the radioactive starting material) be optimized, and that its

TABLE 1

THE SURVIVAL OF MALE ADULT RATS INTUBATED WITH A SINGLE ORAL DOSE OF 2,4-DIMETHYLHEPTANOIC ACID OR SODIUM 2,4-DIMETHYLHEPTANOATE*

	Dose Level (gm/kg)						
Compound	0**	0.1	1.0	2.5	3.0	7.5	10.0
	%	%	%	%	%	%	%
Sodium 2,4-Dimethyl-heptanoate	100 (5)*	100 (3)	100 (4)	100 (2)	---	---	0 (4)
2,4-Dimethyl-heptanoic Acid	100 (8)	---	---	---	100 (3)	0 (6)	

* Figures in parenthesis refer to number of animals per group.

** These animals were intubated with the same volume of the respective solvents as were the test groups.

synthesis be carried out on a micro-scale. Thus conditions for its synthesis are necessarily different from those employed in preparation of unlabeled 2,4-DMHA. Development of these conditions is in progress with unlabeled reactants.

The general scheme of the synthesis involves condensation of diethyl 2-methylpentylmalonate with radioactive methyl iodide in the presence of sodium ethoxide. Saponification, decarboxylation, and isolation will then be carried out in a manner analogous to that employed for the synthesis of non-radioactive 2,4-DMHA. The synthesis of diethyl-2-methylpentylmalonate has been described in section B, II of this report.

Methyl iodide (14.1 mM, 2 gm) was reacted with diethyl-2-methylpentylmalonate (14.8 mM, 3.44 gm) in the presence of sodium ethoxide (14.1 mM, 0.324 gm Na) in 20 ml of absolute ethyl alcohol. The resulting mixture was stirred at room temperature for three hours and then heated under reflux for 12 hours. The reaction mixture was then saponified and decarboxylated in the usual manner and the resultant acid esterified (ref. 3). Gas chromatographic analysis of the methyl ester on the DEGS column (see section B, II) indicated the presence of the usual mixture of diastereoisomers of methyl 2,4-dimethylheptanoate plus about 17% of methyl 4-methylheptanoate which represented unalkylated starting material.

The synthesis will be repeated in order to determine the yield of 2,4-DMHA and to prevent accumulation of 4-methylheptanoic acid as an end product.

V. Determination of Certain Monocarboxylic and Polycarboxylic Acids

A gas chromatographic procedure for the separation of the methyl esters of a number of monocarboxylic and polycarboxylic acids has been established. Esterification of the acids has been carried out successfully and routinely with diazomethane by means of the procedure of Roper and Ma (ref. 3). The esters which have been studied are listed in Table 2. Fumaric acid was not esterified by this procedure.

Gas chromatographic separation of all of the esters listed in Table 3 has been achieved on two different columns. A 1-meter column containing 10% Carbowax 4000 on Chromosorb P, 60/80 mesh, at 150°C brought about the separation shown in Table 3. All the compounds tested, with the exception of dimethyl glutarate and dimethyl 2-methylglutarate, were separated under these conditions.

Improved resolution and particular improved separation of the lower boiling esters was achieved (see Table 4) by using a 1-meter,

TABLE 2

COMPOUNDS STUDIED DURING DEVELOPMENT OF A GAS CHROMATOGRAPHIC METHOD FOR ANALYSIS OF METHYL ESTERS OF CERTAIN MONOCARBOXYLIC AND POLYCARBOXYLIC ACIDS

Monocarboxylic Esters	Dicarboxylic Esters		Tricarboxylic Esters
Methyl 3-hydroxybutyrate	Dimethyl oxalate	Dimethyl 2-methylmalonate	Trimethyl citrate
Methyl 4-methylheptanoate	Dimethyl malonate	Dimethyl 2-methylsuccinate	
Methyl 2,4-dimethyl-heptanoate	Dimethyl glutarate	Dimethyl 2-methylglutarate	
	Dimethyl adipate	Dimethyl 2-hydroxysuccinate Dimethyl malate	
	Dimethyl sebacate		

TABLE 3

GAS CHROMATOGRAPHIC SEPARATION OF CERTAIN METHYL ESTERS ON THE CARBOWAX COLUMN AT 150°C

Ester	Relative Retention Time*
Methyl 2,4-dimethylheptanoate	0.378
Dimethyl oxalate	0.440
Dimethyl 2-methymalonate	0.475
Dimethyl malonate	0.598
Dimethyl 2-methylsuccinate	0.890
Dimethyl succinate	1.00
Dimethyl glutarate	1.49
Dimethyl 2-methylglutarate	1.49
Dimethyl adipate	2.48
Dimethyl 2-hydroxysuccinate	5.15
Dimethyl sebacate	14.4
Trimethyl citrate	24.8

* Relative to methyl succinate (t_r = 4.1 min.).

TABLE 4

GAS CHROMATOGRAPHIC SEPARATION OF CERTAIN METHYL ESTERS ON THE DEGS COLUMN AT 100°C

Ester	Relative Retention Time*
Dimethyl oxalate	0.383
Dimethyl 2-methylmalonate	0.461
Dimethyl malonate	0.651
Dimethyl 2-methylsuccinate	0.804
Dimethyl succinate	1.00
Dimethyl 2-methylglutarate	1.49
Dimethyl glutarate	1.68

* Relative to methyl succinate (t_r = 22.4 min.).

10% diethyleneglycol succinate (DEGS) on 60/65 mesh, acid washed, Chromosorb P column at 100°C.

On the basis of the above results, it was clear that an efficient method for the analysis of a number of monocarboxylic and polycarboxylic acids of interest had been developed. Further refinement of the method is not practical until knowledge of the acids actually obtained as metabolic products becomes available.

VI. Analysis of Urine Acids

Since it is anticipated that metabolic products of 2,4-dimethylheptanoic acid may include acids, preliminary investigation of methods of the isolation of acids from urine seemed warranted. It was also of interest to determine the degree of difficulty of obtaining a gas chromatographic pattern of the methyl esters of the acids thus isolated.

Urine (30 ml), collected over a period of three days from a 400-gm rat and stored at 0°C, was brought to boiling and immediately filtered through SS#597 filter paper. The filtrate was concentrated to a volume of 5 ml in a rotary evaporator at 30°C. The concentrate was saturated with sodium chloride, adjusted to pH 1 with concentrated HCl, transferred to a continuous liquid-liquid extractor, and extracted with ether for 24 hours. The ether extract was dried over anhydrous sodium sulfate and the ether removed in a stream of dry air. The resulting brownish semi-solid residue was dissolved in 1 ml of ether, transferred to a 1/2-dram vial and esterified with diazomethane. The esters thus obtained were chromatographed on the Carbowax 4000 column at 150°C. A total of thirteen separate peaks was observed. No attempt was made to identify these peaks since only the general efficacy of the method was being tested.

As a result of these investigations, it was clear that a complex mixture of acidic components could be isolated in a relatively simple manner from rat urine, and that gas chromatography offered a convenient and promising method for its separation.

SECTION C. ANIMAL EXPERIMENTATION

I. Introduction

The objectives of the animal experimentation phase of this investigation were to establish a metabolic basis for the evaluation of the suitability of potential high-energy dietary components. These studies were concerned with the determination of the metabolic caloric density of these compounds as well as the evaluation of the physiological and nutritional effects of feeding high-energy dietary components.

In the approach that has been followed, the animal studies were divided into the two general areas as follows:

1. Determination of the variables which may affect the reliability and sensitivity of the M.I.T. bio-assay for caloric density.

2. Investigation of the physiological and nutritional effects as well as the metabolism of energy-yielding compounds and the factors which may influence their utilization.

A total of 8 animal experiments were conducted during the period covered by this report. These are classified and discussed in the following order:

1. Caloric bio-assay, Experiments One to Three
2. Utilization and metabolism, Experiments Four to Seven
3. New compounds, Experiment Eight

II. Report on Eight Animal Experiments

The eight whole-animal studies which have been conducted during this report period are described below:

Experiment One

EFFICACY OF THE M.I.T. BIO-ASSAY FOR CALORIC DENSITY USING A DIFFERENT STRAIN OF ANIMALS--CAESAREAN DERIVED "SPECIFIC PATHOGEN FREE" RATS

Introduction and Purpose

In order to achieve more uniform experimental results and to help control disease variables whenever possible, the M.I.T. Animal Laboratory has converted to the use of Caesarean-derived (C.D.) strains of animals which are practically pathogen free. These animals cost more than regular Sprague-Dawley rats, but the extra expense has been justified by healthier animals and more

uniform responses to experimental treatment. In addition, it was soon noted that the C.D. animals grew faster than conventional rats and, therefore, may have different nutritive requirements during the early growth period in which their bacterial flora is being fully developed.

The concept that the C.D. animals may have unique nutritive requirements raised the question of how these animals would respond to the M.I.T. bio-assay for caloric density. Therefore, an experiment was conducted in which the caloric assay procedure was employed with C.D. weanling rats fed known test substances in the form of fat (oleic acid), carbohydrate (sucrose), and protein (lactalbumin).

Experimental Procedure

An essential feature of the M.I.T. bio-assay for caloric density is that animals on the basal unsupplemented diet must lose weight during the 1-week feeding period. Other groups are concurrently fed the basal diet plus graded amounts of supplement. Thus, the growth rate should be directly proportional to the caloric content of the supplement.

Information available on C.D. rats indicated that they had a lower requirement for calories than conventional animals. Therefore, as shown in Table 5, the caloric content of the basal diet was made as low as possible by limiting the amount of corn oil to 1%, just enough to meet linoleic acid requirements. Then, by carrying out a feeding test with various amounts of the basal diet, it was determined that a daily feeding of 8 gm of the basal diet for a 1-week period to animals weighing approximately 46 gm would result in a weight loss of approximately 5 gm. Having established the basal conditions, the formulation of other diets was a matter of adding the test substances as supplements to the basal ration in progressively increasing amounts.

Ten groups of 10 animals each, a total of 100 animals, were used in this study. The animals were weanling Caesarean-derived male rats obtained from the Charles River Laboratories "pathogen free" colony. They were housed in the M.I.T. Animal Laboratories under controlled temperature, humidity, and lighting conditions.

Agar gel diets were used in this test in order to minimize food spillage and permit an accurate determination of food consumption. Diet composition is given in Table 5. The animals were arranged in groups which had an average weight of 47 gm and were given diets in the amounts specified below:

TABLE 5

COMPOSITION OF DIETS USED TO DETERMINE THE EFFICACY OF THE M.I.T. BIOASSAY FOR CALORIC DENSITY USING A DIFFERENT STRAIN OF ANIMALS

	All Curves	Lard Std. Curve			Oleic Acid Test		Sucrose Test		Lactalbumin Test	
	Basal	ST-1	ST-3	ST-5	OA-2	OA-4	SU-2	SU-5	LA-2	LA-3
Casein	400	400	400	400	400	400	400	400	400	400
Sucrose	35	35	35	35	35	35	287	671	35	35
Dextrose	65	65	65	65	65	65	65	65	65	65
Dextrin	45	45	45	45	45	45	45	45	45	45
Corn Oil	10	10	10	10	10	10	10	10	10	10
Salts W	40	40	40	40	40	40	40	40	40	40
Vitamin Mixture	10	10	10	10	10	10	10	10	10	10
Choline	4	4	4	4	4	4	4	4	•4	4
Agar	40	40	40	40	40	40	40	40	40	40
Water	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500
Lard	-	64	188	296	-	-	-	-	-	-
Oleic Acid	-	-	-	-	124	252	-	-	-	-
Lactalbumin	-	-	-	-	-	-	-	-	252	380
Total	2149	2213	2337	2445	2273	2401	2401	2785	2401	2529

No.	Diet	Code	Daily Supple. to 8 gm Basal
Group 1	Basal	B	0 gm
" 2	Lard Std. Curve	ST-1	0.24 gm
" 3	" " "	ST-3	0.67 gm
" 4	" " "	ST-5	1.10 gm
" 5	Oleic Acid Test	OA-2	0.46 gm
" 6	" " "	OA-4	0.93 gm
" 7	Sucrose Test	SU-2	0.93 gm
" 8	" "	SU-5	2.37 gm
" 9	Lactalbumin Test	LA-2	0.93 gm
" 10	" "	LA-3	1.41 gm

Results

Good results were obtained in this test showing that the M.I.T. bio-assay for caloric density can be adapted to different strains of animals by making adjustments in the caloric density and amounts of basal diet fed. Growth curves and calculation data are given in Figure 1 and Table 6. A summary of the results obtained follows.

Test Substance	Energy Utilization Index*	Caloric Density per Gram	
		Found	Approx. Theoretical
Lard	17.48	9.0 (Std.)	9.0
Oleic Acid	14.57	7.5	7.8**
Sucrose	7.26	3.7	4.0
Lactalbumin	8.08	4.2	4.0

*Slope of line relating growth with supplementary caloric intake as per Figure 1.

**Based on 9 Cal/gm x 87% utilization.

Summary

As indicated above, the caloric values obtained for oleic acid, sucrose and lactalbumin are rather close to the actual theoretical figures. It must, therefore, be concluded that if the proper procedures are followed the M.I.T. bio-assay for caloric density can be made applicable to different strains of rats.

Experiment Two

SECOND M.I.T. CALORIC BIO-ASSAY EXPERIMENT USING C.D. "SPECIFIC PATHOGEN-FREE" RATS

Introduction and Purpose

In Experiment One, it was established that the M.I.T. bio-assay for caloric density could be successfully adapted to a new strain of C.D. (Caesarean-derived) animals. Nevertheless, before

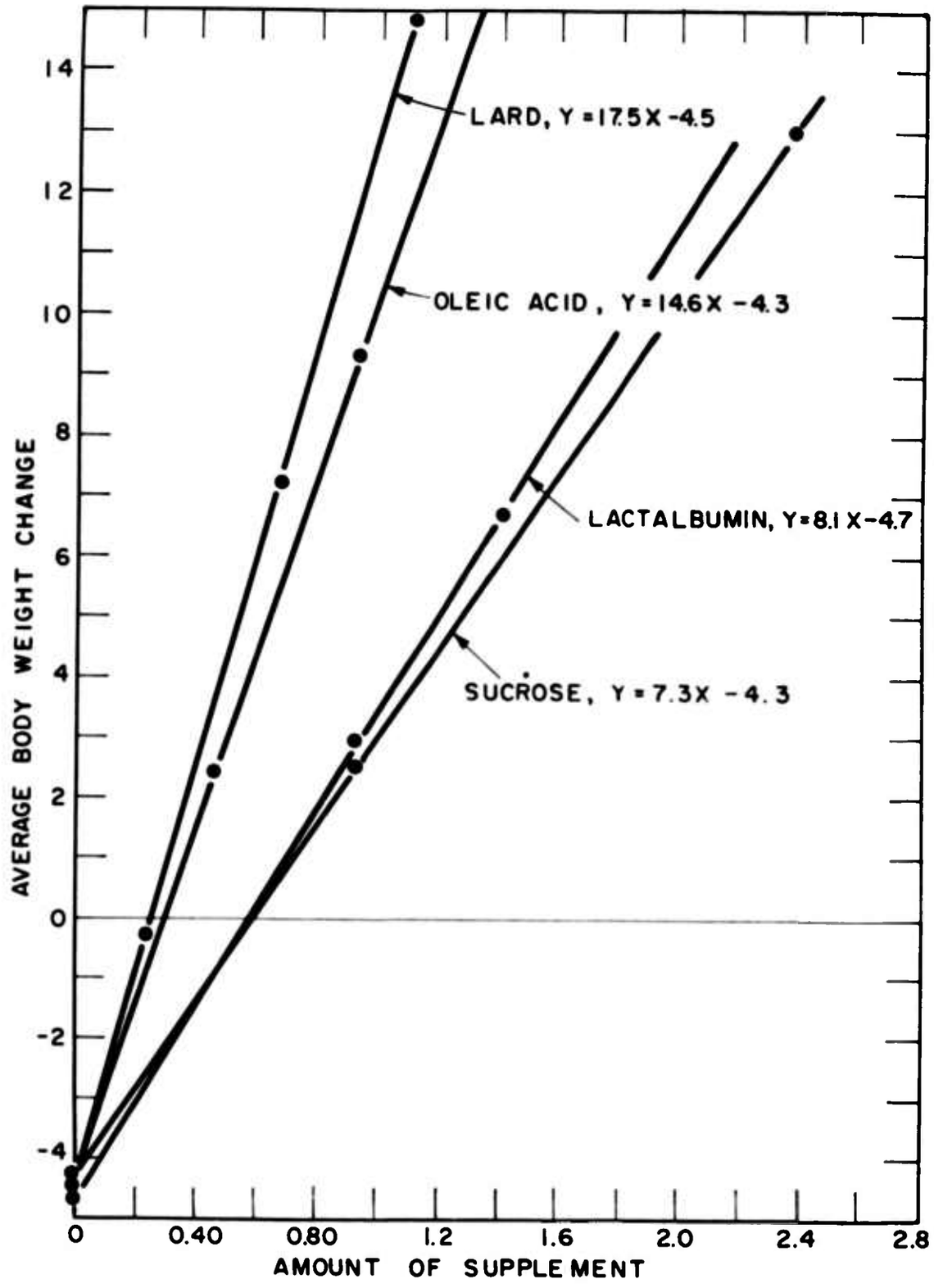

FIG. 1 SLOPES OF LINES RELATING BODY WEIGHT CHANGE WITH AMOUNT OF CALORIC SUPPLEMENT FED

TABLE 6

SUMMARY OF RESULTS AND DATA USED IN CALCULATION OF THE SLOPES AND CALORIC DENSITIES OF TEST MATERIALS SHOWN IN FIGURE 1

	Suppl. Fed	Body Wt. Change	Corrected Body Wt. Change*
	gm	gm	
Lard Std. Curve			
Basal	0	-4.8	-4.5
ST-1	0.24	-0.3	-0.3
ST-3	0.67	7.8	7.2
ST-5	1.10	14.4	14.8
Oleic Acid Curve			
Basal	0	-4.8	-4.3
OA-2	0.46	3.4	2.4
OA-4	0.93	8.9	9.3
Sucrose Curve			
Basal	0	-4.8	-4.3
SU-2	0.93	3.4	2.5
SU-5	2.37	12.6	13.0
Lactalbumin Curve			
Basal	0	-4.8	-4.7
LA-2	0.93	3.3	2.9
LA-3	1.41	6.4	6.7

* These values were determined by the method of least square and were used to locate the lines of best fit shown in Figure 1.

accepting the method, it was decided to repeat the original experiment and also to study a number of other compounds of known caloric density.

Experimental Procedure

Eighteen groups of 10 animals each, a total of 180 animals, were used in this study. The animals were weanling Caesarean-derived (C.D.) rats obtained from the Charles River Laboratories "specific pathogen-free" colony. The animals differed from those used in the previous caloric bio-assay in that they averaged about 4 gm heavier and had a wider range in their individual weights.

As in the previous caloric experiment, agar gel diets were fed in order to permit an accurate allotment of food and to minimize food spillage. Diet composition is given in Table 7. The experimental treatments are outlined in Table 8.

Results

The results obtained in this test were not as good as those reported for the previous caloric bio-assay. As shown in Table 8, growth, as was to be expected, always increased with the addition of larger amounts of supplement. However, the calculated Energy Utilization Indices are higher in this test than those obtained in the earlier test with the exception of that of the standard lard curve. For this reason, caloric densities (Table 9) tended to be greater than the maximum theoretical values. This might have been a function of the higher initial weights of these animals as compared to those of Experiment One, as well as the greater range in initial weights of the animals of the test. The influence of these factors may have resulted in apparently excessive body weight gains which would then produce increased energy utilization indices.

Summary

Results obtained from a second caloric bio-assay using C.D. rats were generally higher than theoretical caloric values. It is thought that the animals used varied too much in initial weight to give precise caloric values. This was studied further.

Experiment Three
FACTORS AFFECTING THE EFFICACY OF THE M.I.T. BIO-ASSAY FOR CALORIC DENSITY

Introduction

This experiment was designed to study the influence of initial body weight of rats on the efficiency of the M.I.T. caloric bio-assay.

TABLE 7

COMPOSITION OF DIETS USED IN SECOND M.I.T. CALORIC BIOASSAY
EXPERIMENT WITH C.D. "PATHOGEN-FREE" ANIMALS

	Basal*	Lard Std. Curve ST-1	ST-3	ST-5	Oleic Acid OA-2	OA-4	Sucrose SU-2	SU-5	Lactalbumin LA-2	LA-3	Dried Whole Egg WE-1	WE-2	WE-3	Metrecal M-1	M-2	M-3	1,3-Butanediol BD-2	Pelargonic Acid PA-1
	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm
Casein	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400	400
Sucrose	35	35	35	35	35	35	252	636	35	35	35	35	35	35	35	35	35	35
Dextrose	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65
Dextrin	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
Corn Oil	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Salts W	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Vitamin Mix	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Choline Chloride	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Agar	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Water	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500
Lard	--	64	188	296	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Oleic Acid	--	--	--	--	124	252	--	--	--	--	--	--	--	--	--	--	--	--
Lactalbumin	--	--	--	--	--	--	--	--	252	380	--	--	--	--	--	--	--	--
Dried Whole Egg	--	--	--	--	--	--	--	--	--	--	124	188	380	--	--	--	--	--
Metrecal	--	--	--	--	--	--	--	--	--	--	--	--	--	252	380	636	--	--
1,3-Butanediol	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	188	--
Pelargonic Acid	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	124
TOTAL	2149	2213	2337	2445	2273	2401	2366	2750	2401	2529	2273	2337	2529	2401	2529	2785	2337	2273

*The basal diet serves as ST-0, OA-0, SU-0, LA-0, WE-0, M-0, BD-0 and PA-0.

TABLE 8
SUMMARY OF RESULTS AND DATA USED IN CALCULATION OF SLOPES AND CALORIC DENSITIES OF TEST MATERIALS

Group	Daily Suppl. to 8 Gm Basal	Total Amt. Fed Daily	Body Wt. Change	Corrected Body Wt. Change*
	gm	gm	gm	gm
LARD STD. CURVE				
B	0	8.0	-9.0	-5.8
ST-1	0.24	8.3	1.3	-1.5
ST-3	0.67	8.7	8.9	6.3
ST-5	1.10	9.1	11.9	14.0
OLEIC ACID CURVE				
B	0	8.0	-9.0	-7.9
OA-2	0.46	8.5	2.4	0.3
OA-4	0.93	8.9	7.7	8.7
SUCROSE CURVE				
B	0	8.0	-9.0	-8.5
SU-2	0.93	8.9	0.2	0.8
SU-5	2.37	10.4	10.9	11.2
LACTALBUMIN CURVE				
B	0	8.0	-9.0	-8.8
LA-2	0.93	8.9	2.2	1.3
LA-3	1.41	9.4	6.0	6.6
DRIED WHOLE EGG CURVE				
B	0	8.0	-9.0	-8.2
WE-1	0.46	8.5	-0.6	-1.8
WE-2	0.67	8.7	1.4	1.2
WE-3	1.41	9.4	11.2	11.5
METRECAL				
B	0	8.0	-9.0	-7.5
M-1	0.93	8.9	1.5	0.8
M-2	1.41	9.4	7.5	5.0
M-3	2.37	10.4	11.9	13.6
1,3-BUTANEDIOL				
B	0	8.0	-9.0	-9.0
BD-2	0.67	8.7	-0.5	-0.6
PELARGONIC ACID				
B	0	8.0	-9.0	-9.1
PA-1	0.46	8.5	4.1	4.1

* Determined by the method of least squares.

TABLE 9

ENERGY UTILIZATION INDEX AND CALORIC DENSITY OF TEST SUBSTANCES

Test Substance	ENERGY UTIL. INDEX*		CALORIC DENSITY PER GRAM		
	Expt. No. 1	Expt. No. 2	Expt. No. 1	Expt. No. 2	Approx. Theoretical
Lard	17.5	18.0	9.0 (Std.)	9.0 (Std.)	9.0
Oleic Acid	14.6	17.8	7.5	8.9	7.8**
Sucrose	7.3	8.3	3.7	4.2	4.0
Lactalbumin	8.1	10.9	4.2	5.5	4.0
Dried Whole Egg	-	14.0	-	7.0	5.9
Metrecal	-	8.9	-	4.5	4.0
1,3-Butanediol	-	12.6	-	6.3	6.5***
Pelargonic Acid****	-	-	-	-	7.5****

* Slope of line relating growth with supplementary caloric intake.
** Based on 9 Cal./g. x 87% utilization.
*** Based on previous estimates.
**** Values obtained too high to be realistic and, therefore, not reported.

Experimental Procedure

The diets and procedures used were similar to those used in Experiment Two. There were two groups of animals with close average initial weights of 40 and 45 gm. Both the 40-and 45-gm groups of rats were further sub-divided and received diets as follows:

Groups		Suppl. (gm)	Amt. Fed (gm)	Ave. Initial Body Wts. 40 Gp. (gm)	45 Gp. (gm)
1. Basal	B	0	8.0	40.6	45.4
2. Lard Std.	ST-1	0.24	8.3	40.6	45.4
3. Lard Std.	ST-3	0.67	8.7	40.6	45.4
4. Lard Std.	ST-5	1.10	9.1	40.5	45.3
5. Sucrose	SU-2	0.93	8.9	40.5	45.3
6. Sucrose	SU-5	2.37	10.4	40.5	45.3
7. Oleic Acid	OA-3	0.67	8.7	40.5	45.3
8. Oleic Acid	OA-5	1.10	9.1	40.5	45.3

Results and Summary

As shown in Table 10, the results clearly show the superiority of 45-gm animals. With the heavier animals, there were fewer "off points" and less need for "corrections." This test should be repeated using animals with average initial weights of 50 and 55 gms.

Experiment Four
COMPARATIVE UTILIZATION AND METABOLISM OF OCTANOIC ACID (EVEN-CARBON, C_8) AND NONANOIC ACID (ODD-CARBON, C_9) IN HIGH-FAT DIETS

Introduction and Purpose

Previous experiments led to the development of the interesting postulate that it may be theoretically possible to increase the caloric density of diets by substituting odd-chain fatty acids for carbohydrate without producing excessive ketosis. One of the odd-carbon fatty acids, which has shown some promise as a limited carbohydrate substitute, is pelargonic acid (nonanoic acid). This compound is of intermediate chain length (9 carbons) and has a caloric density, according to the M.I.T. biological assay, of approximately 7.0 to 7.5 Cal/gm when fed at low to intermediate dietary levels.

Investigations with pelargonic acid revealed several disadvantages. The compound appeared to be relatively unpalatable and

TABLE 10

SUMMARY OF GRADED BODY WEIGHT CALORIC TEST: EFFECT OF USING ANIMALS WITH INITIAL WEIGHTS OF 40 AND 45 GRAMS*

	40-Gram Rats				45-Gram Rats			
	Slope (E.U.T.)		Cal/gm		Slope (E.U.I.)		Cal./gm	
	uncorr-ected	corr-ected	uncorr-ected	corr-ected	uncorr-ected	corr-ected	uncorr-ected	corr-ected
Lard	19.7	20.8	9.0	9.0	19.7	-	9.0	9.0
Sucrose	7.4	7.8	3.4	3.4	7.8	7.8	3.6	3.6
Oleic Acid	17.0	17.2	7.8	7.4	20.1	20.2	9.2	9.2

Group Values

	40-Gram Rats			45-Gram Rats		
	Change in Body Weight			Change in Body Weight		
	uncorr-ected	corr-ected	Off Points	uncorr-ected	corr-ected	Off Points
	gm	gm		gm	gm	
Basal	-10.5	-	None	-12.6	-	None
ST-1	-4.8	-5.4	1	-7.2	-	None
ST-3	+4.1	+4.1	2	+1.1	-	None
ST-5	+11.3	+12.4	1	+9.3	-	None
SU-2	+0.6	+1.4	2	-2.2	-2.9	1
SU-5	+7.6	+8.7	1	+6.3	-	None
OA-3	+2.4	+3.7	2	+2.8	+3.5	1
OA-5	+8.0	-	None	+9.3		None

* There were five animals per each test level.

was best utilized if used at dietary levels no greater than 10%. The more desirable higher levels of usage produced a number of adverse effects. Determining the reasons for the adverse effects, however, was difficult because of limited knowledge concerning the metabolism of odd-carbon fatty acids which are generally not present in natural fats.

It appeared, therefore, that some studies should be carried out on the metabolism of pelargonic acid, especially when incorporated in high-fat diets. For comparative purposes, octanoic acid, an 8-carbon compound, was selected as a companion fatty acid for this test. The purpose of this test was to compare the utilization and metabolism of odd-carbon and even-carbon fatty acids of intermediate chain length.

Experimental Procedure

Four groups of 20 rats each, a total of 80 animals, were used in this study. The animals were weanling male Sprague-Dawley derived rats obtained from the Charles River Breeding Laboratory, Wilmington, Massachusetts. They were housed in the M.I.T. Animal Laboratories under controlled temperature, humidity and lighting conditions.

Agar gel diets were used in these tests because these diets reduce food spillage and permit accurate measurement of food consumption. Diet composition is given in Table 11. The approximate caloric content of the diets used is shown in Table 12. The four experimental groups were:

1. 10% Fat Control
2. 35% Fat Control
3. 35% Fat + 20% Octanoic Acid
4. 35% Fat + 20% Nonanoic Acid (Pelargonic Acid)

The percentages of fat and octanoic and nonanoic acid indicated above are on a dry diet basis. High levels of fat (35%) were incorporated into the experimental diets in order to attain maximum caloric density and to accentuate the effects of the octanoic and pelargonic acids. As shown in Tables 11 and 12, the fatty acid-containing diets contained 55% fatty materials, composed of 35% natural fat from lard and corn oil, plus 20% octanoic or pelargonic acid. In these diets, approximately 52% of the total calories were derived from natural fat and 23% from the fatty acids. Thus, in groups 3 and 4, approximately 75% of the total calories in the diets were obtained from fatty sources.

Food intake was measured daily, and the a..imals weighed weekly during the experimental period of 21 days. Upon termination of

TABLE 11

COMPOSITION OF DIETS USED TO DETERMINE THE COMPARATIVE UTILIZATION AND METABOLISM OF OCTANOIC ACID (EVEN-CARBON, C_8) AND NONANOIC ACID (ODD-CARBON, C_9) IN HIGH-FAT DIETS*

Ingredients	10% Fat	35% Fat	35% Fat + 20% Octanoic Acid	35% Fat + 20% Nonanoic Acid
	gm	gm	gm	gm
Casein	220	220	220	220
Sucrose	198	115	48	48
Dextrose	395	228	95	95
Salt Mixture W	40	40	40	40
Lard	60	200	200	200
Agar	35	35	35	35
Corn Oil	40	150	150	150
Vitamin Mixture	10	10	10	10
Choline Chloride	2	2	2	2
Octanoic Acid	-	-	200	-
Nonanoic Acid	-	-	-	200
Water	1000	1000	1000	1000
Total	2000	2000	2000	2000

* Diets contain 10% fat or 35% fat plus 20% octanoic or nonanoic acid on a dry diet basis, that is without the 100% addition of water in the diet.

TABLE 12

CALORIC DISTRIBUTION OF EXPERIMENTAL DIETS

Ingredients	10% Fat		35% Fat		35% Fat + 20% Octanoic Acid		35% Fat + 20% Nonanoic Acid	
	Cal.	%	Cal.	%	Cal.	%	Cal.	%
Protein	880	21.0	880	16.2	880	14.6	880	14.6
Carbohydrate	2415	57.5	1412	26.0	612	10.1	612	10.1
Fat	900	21.5	3150	57.8	3150	52.2	3150	52.2
Octanoic Acid*	-	-	-	-	1400	23.1	-	-
Nonanoic Acid*	-	-	-	-	-	-	1400	23.1
Total	4195	100.0	5442	100.0	6042	100.0	6042	100.0
Cal./gm	4.2		5.4		6.0		6.0	

* Octanoic acid and nonanoic acid were assumed to yield 7 Cal./gm when metabolized.

the experiment at 3 weeks, the animals were fasted overnight. Then each of the four groups of animals was further divided into three sub-groups. At 0, 1, and 3 hours after administration of 5 gm of diet to the fasting rats, one of the three sub-groups of each test groups was sacrificed.

At the appropriate time after feeding of all 5 gm of diet, animals were sacrificed by decapitation and the blood collected. After clotting, the serum was separated and quickly frozen until the time of analysis. Livers from each animal were removed, weighed, and quickly frozen until analyzed.

The metabolic variables measured at 0, 1, and 3 hours after ingestion of 5 gm of diet were serum glucose, liver glycogen and total serum ketone bodies. Serum glucose was determined by the micro glucose oxidase method of Cawley, et al. (ref. 11). The procedure employed for the assay of liver glycogen was reported by Kemp and Van Heoingen (ref. 12). Total serum ketone bodies were analyzed by a modification of the method devised by Lyon and Bloom (ref. 13).

Results

Feeding Study

The animal feeding results, summarized in Table 13, show that diets containing either octanoic or pelargonic acid did not produce the normal rate of growth obtained with the unsupplemented diets containing 10% and 35% fat. While body weight gains in the unsupplemented 10% and 35% fat-containing diets were fairly equivalent, weight gains markedly decreased when the diets contained either octanoic or pelargonic acid. The least growth was obtained when animals were fed the pelargonic acid diet. This trend, as indicated by the weekly body weights given in Table 14, appeared during the first week on test and was maintained throughout the course of the experiment. There is no doubt that octanoic acid and pelargonic acid diets failed to support good growth in the test rats. These results can be best visualized by referring to the growth curves given in Figure 2.

Food consumption was highest on the 10% fat diet and progressively decreased with the addition of fat (35% fat diet), octanoic acid, and pelargonic acid, respectively. In this experiment, as in previous tests, there was a palatability problem with the pelargonic acid diet. It appeared that octanoic acid was also not well accepted by the rat.

As may be expected, the best food efficiency was obtained in rats consuming the 35% fat diet. Intermediate food efficiencies

TABLE 13

SUMMARY OF WEIGHT GAINS, FOOD CONSUMPTION, FOOD EFFICIENCY, AND APPROXIMATE URINARY KETOSIS AFTER THREE WEEKS OF FEEDING OCTANOIC ACID AND NONANOIC ACID IN HIGH-FAT DIETS

Group	Treatment	Ave. Wt. Gain	Ave. Food Consumed	Ave. Food Efficiency	Approx. Degree of Urinary Ketosis*
		gm	gm		3 wks.
1	10% Fat	106.5	438.6	24.1 ± 0.5	None
2	35% Fat	102.1	370.2	27.7 ± 0.5	Small
3	35% Fat + 20% Octanoic Acid	62.7	296.5	21.1 ± 0.7	Moderate
4	35% Fat + 20% Nonanoic Acid	35.7	220.9	16.2 ± 0.8	Moderate

* Determined by testing the urine with Ketostix.

TABLE 14

AVERAGE WEEKLY WEIGHTS OF EXPERIMENTAL ANIMALS FED DIETS CONTAINING VARIOUS LEVELS OF FAT AND SUPPLEMENTED WITH OCTANOIC AND NONANOIC ACIDS

Group	Treatment	Body Weight			
		0 Wk.	1st Wk.	2nd Wk.	3rd Wk.
		gm	gm	gm	gm
1	10% Fat	56.2 ± 1.0	90.6 ± 2.0	122.5 ± 3.4	162.7 ± 4.6
2	35% Fat	56.2 ± 1.0	90.4 ± 2.4	120.3 ± 3.5	159.8 ± 4.3
3	35% Fat + 20% Octanoic Acid	56.2 ± 0.9	80.4 ± 1.4	99.6 ± 1.9	118.8 ± 2.3
4	35% Fat + 20% Nonanoic Acid	56.2 ± 0.9	68.2 ± 1.2	80.3 ± 1.7	91.9 ± 1.8

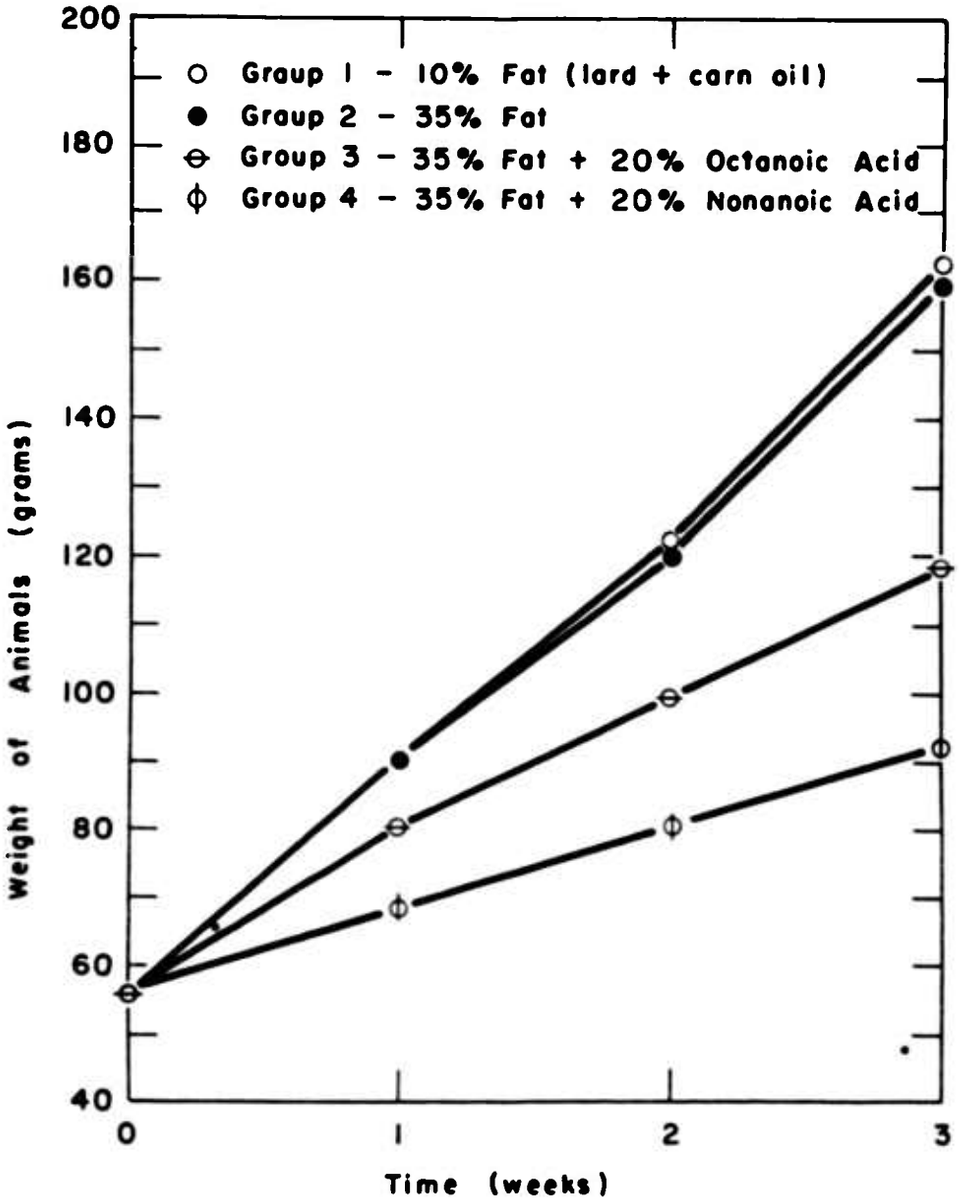

FIGURE 2.

GROWTH OF RATS FED CONTROL AND HIGH-FAT DIETS SUPPLEMENTED WITH OCTANOIC AND NONANOIC ACIDS

were obtained in animals consuming the 10% fat diet and the octanoic acid diet. The poorest food conversion resulted from feeding pelargonic acid. In fact, the food efficiency on the octanoic acid was significantly greater ($p = 0.05$) than that obtained with pelargonic acid.

Metabolic Study

As mentioned previously, blood glucose, liver glycogen, and total serum ketone bodies were determined at 0-, 1-, and 3-hour intervals after feeding 5 gm of diet to fasting animals. A summary of the glucose, glycogen, and ketone body data is presented in Table 15.

Figure 3 shows the changes in blood glucose values over the 3-hour experimental period. Animals fed the unsupplemented 10% fat and 35% fat diets had normal values of approximately 100 mg % at zero time, while glucose levels of animals fed the fatty acid-containing diets were approximately 65 mg %. Nevertheless, the blood glucose values in all groups approximately doubled during the first hour and remained at those values during the next 2 hours. Animals on the two unsupplemented diets attained blood glucose levels of approximately 190 mg %, while those on the acid-containing diets had glucose values of approximately 120 mg %. These increases in blood glucose appear to correlate well with the amount of carbohydrate in the diets.

Liver glycogen values did not continue to follow the pattern found in the blood glucose results. When percentage increases in liver glycogen were considered, the responses of the 10% fat and the pelargonic acid-fed groups were quite similar. On the other hand, the animals fed diets containing 35% fat or octanoic acid responded in a similar manner. These similarities in direction but not in degree are readily seen by examination of Figure 4. This figure shows that both the animals fed 10% fat diets and those fed pelargonic acid continued to store liver glycogen during the whole 3-hour observation period. On the other hand, in the 35% fat and the octanoic acid groups, liver glycogen reached a peak in 1 hour and declined at 3 hours.

It is interesting to note that the livers from the animals fed pelargonic acid contained the least amount of glycogen. Another interesting point is the sizable increase in liver glycogen obtained in 1 hour in the rats fed octanoic acid. Nevertheless, the data indicate that the unsupplemented 10% fat and 35% fat diets were the most glycogenic when the storage of glycogen is considered on an absolute basis.

Figure 5 illustrates the changes in total serum ketone bodies at 0, 1, and 3 hours after the fasted rats had consumed 5 gm of

TABLE 15

CHANGE IN BLOOD GLUCOSE AND LIVER GLYCOGEN OF FASTED ANIMALS FOLLOWING THE INGESTION OF 5 GRAMS OF DIET*

Group	Treatment	Ave. Blood Glucose (Hours)			Ave. Liver Glycogen (Hours)		
		0	1	3	0	1	3
		mg. %	mg. %	mg. %	mg. %	mg. %	mg. %
1	10% Fat	121 ± 18.1	192 ± 17.4	193 ± 9.2	148 ± 19.6	349 ± 12.5	570 ± 13.5
2	35% Fat	86 ± 16.3	190 ± 13.9	192 ± 13.9	241 ± 14.5	658 ± 18.5	424 ± 12.3
3	35% Fat + 20% Octanoic Acid	61 ± 5.4	125 ± 10.1	123 ± 9.9	155 ± 15.9	420 ± 11.9	295 ± 11.7
4	35% Fat + 20% Nonanoic Acid	69 ± 1.1	126 ± 14.5	130 ± 6.4	35 ± 6.1	86 ± 13.5	167 ± 10.7

* Performed at the conclusion of the 3-week feeding period.

CHANGE IN TOTAL SERUM KETONE BODIES OF FASTED ANIMALS FOLLOWING THE INGESTION OF 5 GRAMS OF DIET

Group	Treatment	Ave. Total Serum Ketone Bodies (Hours)		
		0	1	3
		mg. %	mg. %	mg. %
1	10% Fat	4.11 ± 0.18	2.39 ± 0.12	2.22 ± 0.12
2	35% Fat	4.45 ± 0.18	3.66 ± 0.16	3.74 ± 0.11
3	35% Fat + 20% Octanoic Acid	5.18 ± 0.15	5.17 ± 0.25	5.63 ± 0.21
4	35% Fat + 20% Nonanoic Acid	4.86 ± 0.20	4.43 ± 0.15	4.27 ± 0.15

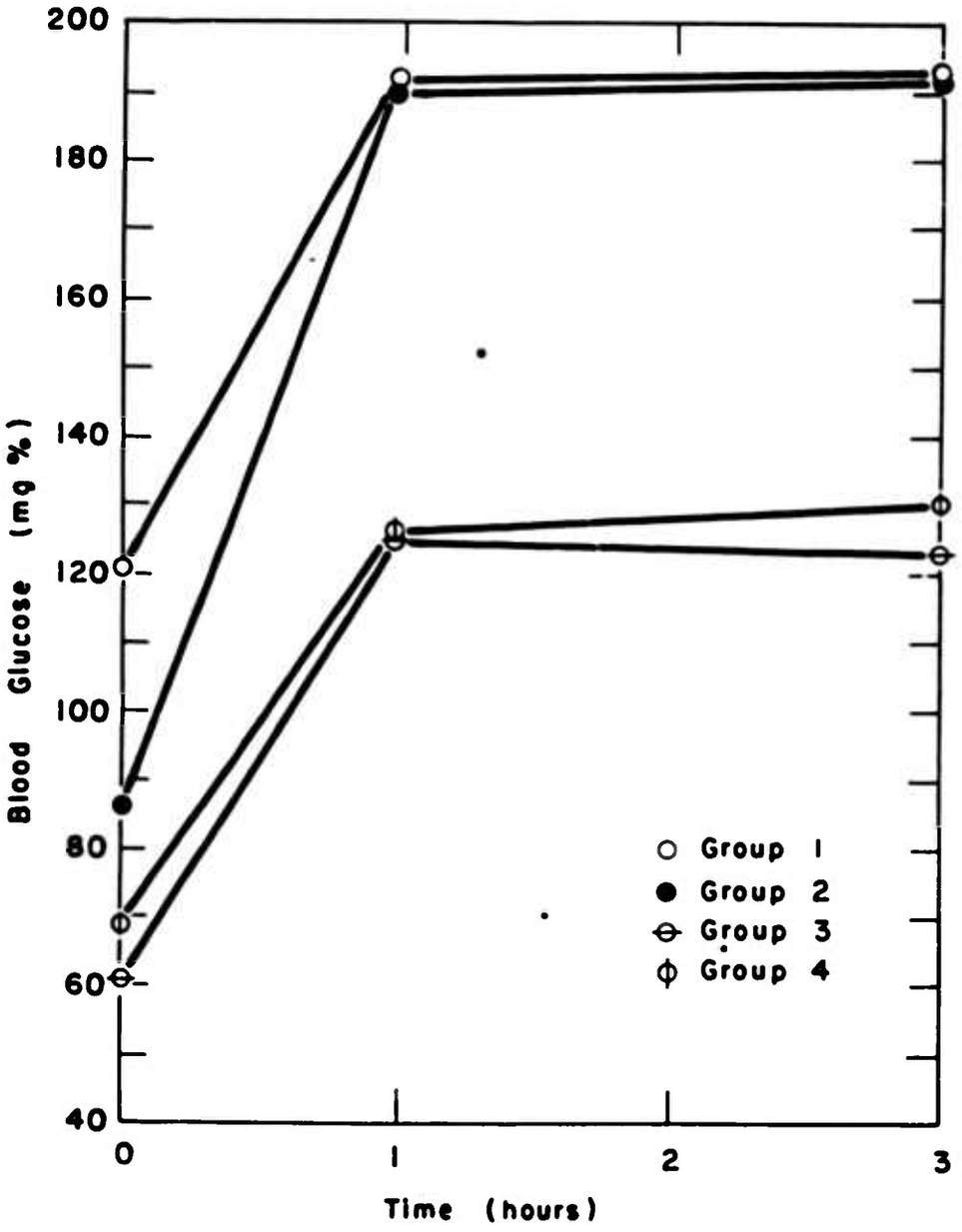

FIGURE 3

CHANGE WITH TIME OF BLOOD GLUCOSE OF FASTED ANIMALS FED 5 GRAMS OF THEIR RESPECTIVE EXPERIMENTAL RATIONS

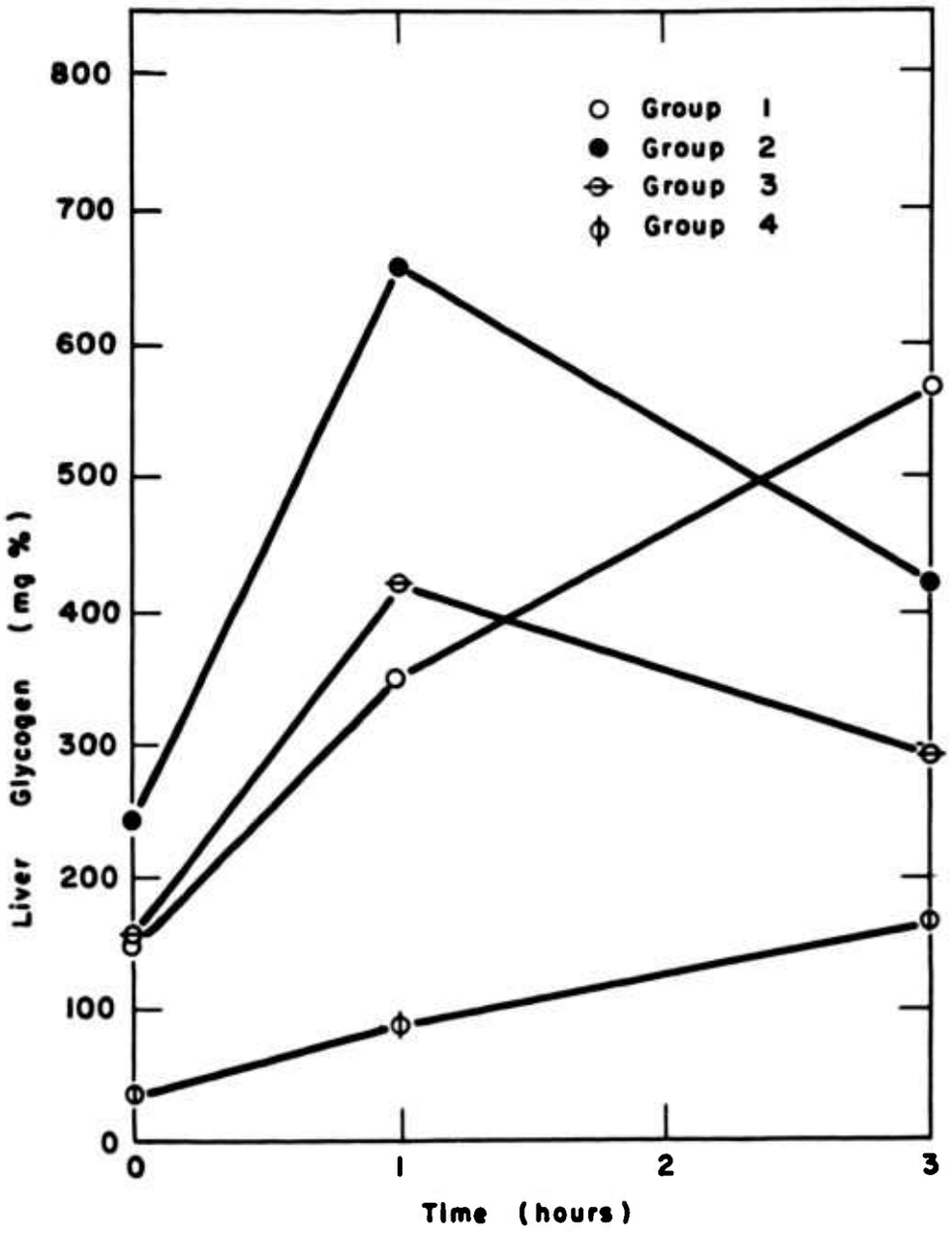

FIGURE 4

CHANGE IN LIVER GLYCOGEN OF FASTED RATS FED 5 GRAMS OF THEIR RESPECTIVE EXPERIMENTAL RATIONS

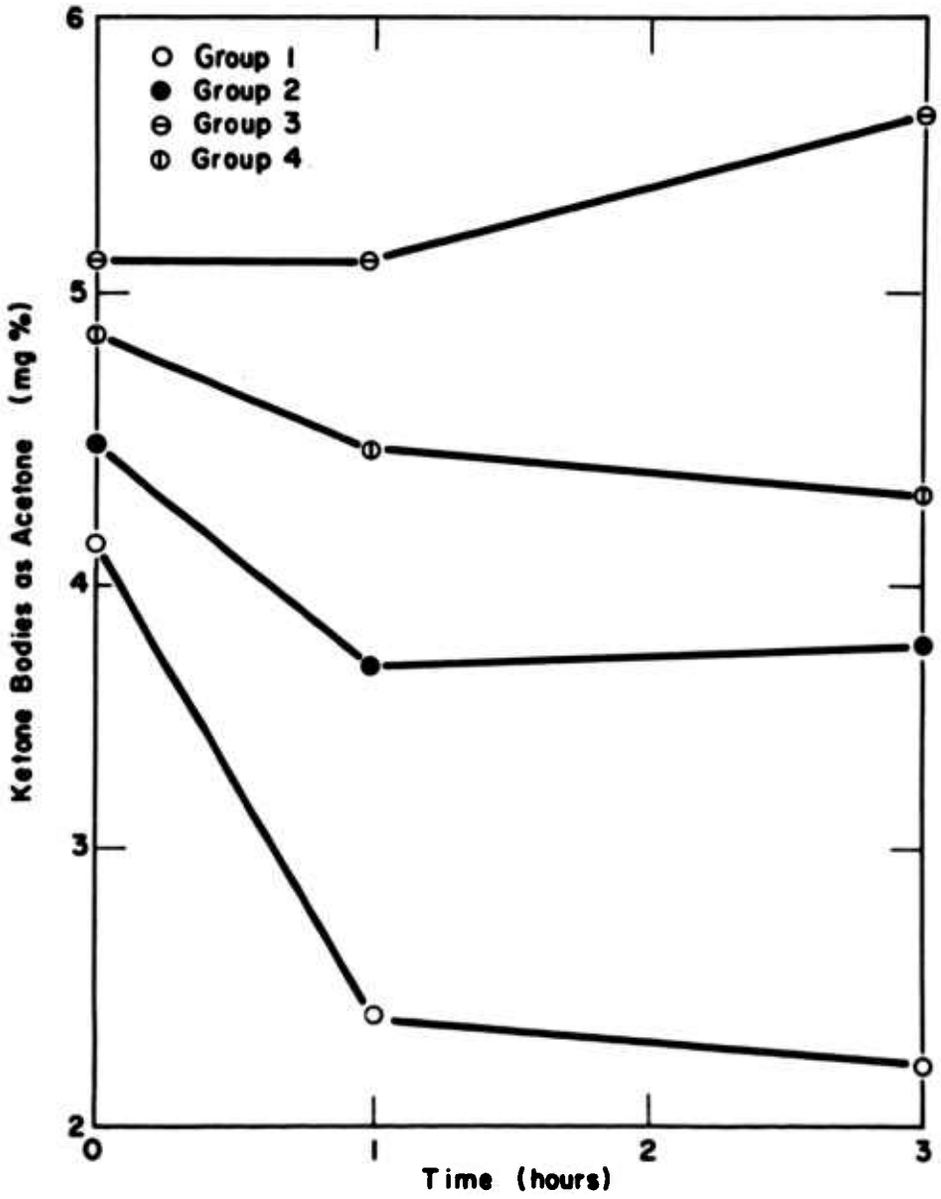

Figure 5

CHANGE WITH TIME OF SERUM KETONE BODIES OF FASTED RATS FED 5 GRAMS OF THEIR RESPECTIVE EXPERIMENTAL RATIONS

diet. As anticipated, the 10% fat diet was least ketogenic followed by the 35% fat diet. Pelargonic acid was next in order of decreasing ketogenicity. Thus, octanoic acid proved to be most ketogenic and was the only treatment which showed an increase in ketosis over the zero time value in 3 hours.

The results of this metabolic study indicate that octanoic acid, an even-carbon fatty acid, and pelargonic acid, an odd-carbon fatty acid, are metabolized differently. This result was not unexpected since beta-oxidation of octanoic acid would produce four 2-carbon fragments while that of pelargonic acid should produce three 2-carbon fragments and one 3-carbon fragment. Then, if 3-carbon fragments entered carbohydrate metabolic pathways, pelargonic acid would be partly glucogenic. However, this theoretical possibility had not been investigated previously with high-fat rations.

The continuous increase in liver glycogen and decrease in total serum ketone bodies noted over the 3-hour observation period appear to confirm the fact that pelargonic acid may be glucogenic. Nevertheless, the failure of sufficient levels of pelargonic acid to be adequately consumed and to support normal growth in rats remain as problems to be solved before this compound can be considered a suitable source of dietary energy. This study does indicate, therefore, that some odd-carbon fatty acid may very well prove to be a satisfactory carbohydrate substitute.

Summary

Pelargonic acid (odd-carbon, C_9) and octanoic acid (even-carbon, C_8) were added at 20% levels to high-fat diets containing 35% corn oil and lard. The diets were fed ad libitum to weanling rats for a period of 3 weeks. Neither diet produced satisfactory weight gains or food efficiencies as compared to an unsupplemented 35% fat diet.

At the termination of the 3-week feeding period, all rats were fasted overnight and then fed 5 gm of their respective diets. Groups of rats were sacrificed at 0, 1, and 3 hours after the feeding and analyses made for blood glucose, liver glycogen and total serum ketone bodies. It was found that both pelargonic acid and octanoic acid diets increased blood glucose but not to the same extent as the unsupplemented diets. Octanoic acid produced greater replenishment of liver glycogen stores than pelargonic acid. Nevertheless, the fact that liver glycogen continued to rise throughout the whole 3-hour observation period in the animals fed pelargonic acid indicated that the compound may, to some extent, be glycogenic. Further evidence for this was obtained by results showing that pelargonic acid was less ketogenic than octanoic acid.

The results obtained appear to support the contention that odd-carbon fatty acids, when fed as a part of an adequate high-fat ration, may be partly glucogenic and that suitable odd-carbon compounds may prove to be capable of replacing a large portion of the dietary carbohydrate.

Experiment Five

EFFECT OF PAIRED FEEDING (EQUALIZED FOOD INTAKE) OF GRADED LEVELS OF 1,3-BUTANEDIOL AND NONANOIC ACID AS CARBOHYDRATE REPLACEMENTS IN HIGH-FAT DIETS

Introduction and Purpose

In Experiment 7 (WADD Technical Report 60-575) and Experiment Four (this report), two potential high-energy materials, 1,3-butanediol and nonanoic acid (pelargonic acid) were fed at graded levels as carbohydrate replacements in "high-fat" diets. The diets were designated as "high-fat" diets because they contained 25% or 35% fat (dry basis), while the rat is normally fed diets containing only 10% fat. The butanediol and nonanoic acid supplements were always added at the expense of the lower-energy carbohydrate rather than the fat in order to attain maximum caloric density.

The results of the previous feeding tests indicated that either 1,3-butanediol or pelargonic acid could be fed only at low dietary levels. High levels, such as 20%, appeared to have a detrimental effect on growth, or food utilization, or both. Nevertheless, even with a 10% level of supplementation, fat plus supplement can make up almost 60% of the total calories in the diet.

From the information obtained in these earlier experiments it was not known why levels higher than 10% of 1,3-butanediol or pelargonic acid were not well accepted metabolically. Of several possible explanations, one was that the reductions in food intake observed with higher levels of the test substances may have resulted in the consumption of insufficient quantities of protein and/or other nutrients. In this instance, the reductions in food intake may be partly related to increases in diet caloric density. On the other hand, the food consumption problem encountered may have been caused by lack of palatability, poor absorption, or toxicity.

In order to help resolve questions of diet acceptance and utilization and to eliminate differences due to the consumption of unequal amounts of food, a paired feeding test was initiated. The purpose of the experiment was to determine the effect of feeding graded levels of 1,3-butanediol and pelargonic acid under conditions of equalized nutrient intake.

Experimental Procedure

Six groups of 10 rats each, a total of 60 animals, were used in this study. The animals were male Sprague-Dawley strain weanlings obtained from the Charles River Breeding Laboratory, Wilmington, Massachusetts. They were maintained in the M.I.T. Animal Laboratories under controlled environmental conditions. Diet composition is given in Table 16. The experimental groups were as follows:

1. 10% Fat Control
2. 25% Fat Control
3. 25% Fat + 10% Pelargonic Acid
4. 25% Fat + 30% Pelargonic Acid
5. 25% Fat + 5% 1,3-Butanediol
6. 25% Fat + 20% 1,3-Butanediol

Since this was a paired-feeding experiment, feeding was controlled in order to equalize food intake. Animals were weighed weekly, at which time urinary ketone body measurements were made. The experimental period was four weeks and three days.

Results

The experimental results of this test are summarized in Table 17.

As indicated in Table 17, average food consumption was essentially the same with all diets. On the other hand, as was expected, caloric intake of the animals increased as the level of fat was raised from 10% (control diet) to 25% (experimental diet) and as the level of supplement increased (10% to 30% P.A. and 5% to 20% B.D.). Body weight gains were better with all of the 25% high fat experimental diets than with the 10% fat control diets. Moreover, the high fat diets containing supplements of either pelargonic acid or 1,3-butanediol supported better growth than the unsupplemented 25% fat diet.

It is also important to note that animals fed diets containing either pelargonic acid or 1,3-butanediol had food efficiencies which were superior to those obtained with the unsupplemented 10% or 25% fat diets. This same trend was also generally evident with average caloric efficiency. The caloric efficiency of animals fed pelargonic acid or 1,3-butanediol was generally similar or better than that of animals which did not receive these supplements.

The data indicate that under conditions of equal food and nutrient intake, dietary supplements of 10% and 30% pelargonic acid and 5% and 20% 1,3-butanediol gave body weight gains and food

TABLE 16

COMPOSITION OF DIETS USED TO STUDY EFFECT OF PAIRED FEEDING OF GRADED LEVELS OF 1,3-BUTANEDIOL AND PELARGONIC ACID AS CARBOHYDRATE REPLACEMENTS IN HIGH-FAT DIETS

Ingredients	1 10% Fat Control	2 25% Fat Control	3 25% Fat + 10% P.A.	4 25% Fat + 30% P.A.	5 25% Fat + 5% B.D.	6 25% Fat + 20% B.D.
	gm	gm	gm	gm	gm	gm
Casein	220	220	220	220	220	220
Sucrose	194	144	111	44	127	77
Dextrose	389	289	222	89	126	156
Lard	60	150	150	150	150	150
Corn oil	40	100	100	100	100	100
Salts W	40	40	40	40	40	40
Liver powder	10	10	10	10	10	10
Vitamin Mixture*	10	10	10	10	10	10
Choline Chloride	2	2	2	2	2	2
Agar	35	35	35	35	35	35
Water	1000	1000	1000	1000	1000	1000
Pelargonic Acid	-	-	100	300	-	-
1,3-Butanediol	-	-	-	-	50	200
Vanilla	-	-	-	-	-	-
Total	2000	2000	2000	2000	2000	2000

APPROXIMATE CALORIC DISTRIBUTION - DRY BASIS*

	Cal.	%Cal.	Cal.	%Cal.	Cal.	%Cal.	Cal.	%Cal.	Cal.	%Cal.	Cal.	%Cal.
Protein	880	21	880	18	880	17	880	15	880	18	880	17
Carbohydrate	2332	57	1732	36	1332	25	532	10	1532	31	932	18
Fat	900	22	2250	46	2250	44	2250	39	2250	45	2250	42
P.A. or B.D.	--	--	--	--	700	14	2100	36	300	6	1200	23
Total Cals.	4112		4862		5162		5762		4962		5262	
Cal./gm	4.11		4.86		5.16		5.76		4.96		5.26	

P.A. - Pelargonic acid, assumed to have 7 Cal./gm in calculations.

B.D. - 1,3-Butanediol, assumed to have 6 Cal./gm in calculations.

* Values adjusted to include calories supplied by liver powder and vitamin mix diluent.

TABLE 17

EFFECT OF PAIRED FEEDING OF GRADED LEVELS OF 1,3-BUTANEDIOL AND PELARGONIC ACID AS CARBOHYDRATE REPLACEMENTS IN HIGH-FAT DIETS

Experiment Group	Ave. Food Consumed	Ave. Cal. Consumed	Ave. Body Wt. Gain	Ave. Food Efficiency	Ave. Cal. Efficiency
	gm	Cal.	gm	%	
10% Fat Control	320	1315	51.3	16.0	3.9
25% Fat Control	319	1550	63.9	20.0	4.1
25% Fat + 10% P.A.	320	1747	73.9	23.1	4.2
25% Fat + 30% P.A.	320	1843	73.1	22.8	4.0
25% Fat + 5% B.D.	320	1587	71.5	22.3	4.5
25% Fat + 20% B.D.	320	1683	70.5	22.2	4.2

P.A. - Pelargonic acid.
B.D. - 1,3-Butanediol.

and caloric efficiencies equal to, and generally better than, unsupplemented controls. Thus, it appears that there are at least two possible explanations for the apparently poor results obtained with high levels of these supplements in previous experiments. These are: (1) the possible reduction of food intake caused by increased caloric density of the diets, and (2) the significant part played by feeding unpalatable materials which are not well accepted by the rat.

Solution of a palatability problem may lie in such techniques as force feeding, encapsulation, or flavor masking. In an earlier experiment (No. 7, WADD Technical Report 60-575), the use of vanillin as a flavor-masker for these compounds was not very successful.

No ketonuria was observed in any of the groups of animals.

Summary

When food intake was equalized, diets containing graded levels of 10% and 30% pelargonic acid and 5% and 20% 1,3-butanediol gave as good growth and food and caloric efficiency as an unsupplemented control diet. This indicates that poor palatability was one major factor in influencing the results obtained in previous tests.

Experiment Six
EQUAL CALORIC FEEDING BY INTUBATION OF LIQUID DIETS CONTAINING CORN OIL ALONE AND WITH 1,3-BUTANEDIOL AND NONANOIC ACID

Introduction

Previously, it has been difficult to conduct a satisfactory equal caloric, comparative feeding of corn oil, 1,3-butanediol and nonanoic acid (pelargonic acid) because of the low palatability of the latter two compounds. Under conditions of ad libitum feeding, in many instances, rats have not consumed sufficient amounts of diets containing either 1,3-butanediol or pelargonic acid to maintain or increase their body weight. However, with the development of liquid diets which can be intubated, the food acceptance, or palatability, problem could be circumvented.

The general objective of this experiment was to determine whether 1,3-butanediol and pelargonic acid could effectively replace lower-energy carbohydrates in high fat diets. The experimental rations used in these tests were designed to be isocaloric, in order that all animals could be intubated with the same total amount of diet. Under these conditions, increases in body weights of all of the experimental groups should be equal if the following assumptions are correct:

1. The estimated caloric densities of both 1,3-butanediol and pelargonic acid are correct.
2. The test compounds are nontoxic.
3. The potential energy of each of the test compounds is biologically available.

Experimental Procedure

A total of 60 animals, divided into 6 equally weighted groups of 10 animals each, was used in the study. The animals were Sprague-Dawley male rats (Charles River Breeding Laboratory) which weighed approximately 87 gm each. They were housed in individual cages in temperature- and humidity-controlled animal quarters. Experimental groups were as follows:*

1. 30% Fat (corn oil)
2. 30% Fat + 20% 1,3-Butanediol
3. 30% Fat + 20% 1,3-Butanediol (protein adjusted)**
4. 30% Fat + 20% Pelargonic Acid
5. 30% Fat + 20% Pelargonic Acid (protein adjusted)**
6. Purina Chow (dry) + Intubated Water (control)

Groups 1 to 5 were uniformly intubated, 1 to 3 times daily, with the same volume of their respective isocaloric diets. The control group (Group 6) was fed dry Purina Chow ad libitum, but was intubated with the same volume of water as the other groups received of liquid diet.

The composition of the diets which, as far as could be determined, were nutritionally adequate for the growing rat, is recorded in Table 18.

The diets were prepared as follows. The lactalbumin, sugars, celluflour, minerals and vitamins were incorporated into a dry mix. A two-day supply of the liquid diets was prepared by the addition of liquids in the form of water, corn oil, 1,3-butanediol, or pelargonic acid. The dry and liquid ingredients were then mixed in a Waring Blendor. Following preparation, the diets were stored at refrigerator temperature.

The diets were fed with the use of a syringe and a ball-pointed stainless steel intubation tube.

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*All percentages are given on a dry weight basis.

**The amount of protein in these rations is equivalent to that of controls to obviate possible differences in growth due to the differences in the quantity of protein.

TABLE 18

COMPOSITION OF DIETS USED IN EQUAL CALORIC FEEDING OF CORN OIL, 1,3-BUTANEDIOL AND PELARGONIC ACID BY INTUBATION

	1 30% Fat	2 30% Fat + 20% B.D.	3 30% Fat + 20% B.D. (H.P.)	4 30% Fat + 20% P.A.	5 30% Fat + 20% P.A. (H.P.)	6 Purina Chow
	gm	gm	gm	gm	gm	
Dry Mix						
Lactalbumin	200	200	222	200	230	Fed Purina Chow ad libitum, but intubated with water.
Sucrose	100	52	47	52	45	
Dextrose	211	107	95	107	91	
Dextrin	100	52	47	52	45	
Cellulose	35	35	35	35	35	
Salts W	40	40	40	40	40	
Vitamin Mixture	10	10	10	10	10	
Choline Chloride	4	4	4	4	4	
Liquids added before use						
Corn Oil	300	300	300	300	300	
1,3-Butanediol	--	200	200	--	--	
Pelargonic Acid	--	--	--	200	200	
Water	500	646	646	709	709	
Total	1500	1646	1646	1709	1709	
CALCULATED COMPOSITION						
Cal./gm or ml.(wet basis)	3.5	3.5	3.5	3.5	3.5	
Protein,% (wet basis)	10.7	9.7	10.8	9.4	10.8	

Approximately equal in caloric content - Groups 1,2,3,4,5.
Approximately equal in protein content - Groups 1,3,5.

B.D. - 1,3-Butanediol; P.A. - Pelargonic Acid; H.P. - Slightly higher protein level, approximately equal to protein content of 30% fat diet (Group 1).

Results

The results, which are summarized in Table 19, indicate that following an adaptation period of one week, caloric utilization of 1,3-butanediol and pelargonic acid approached but did not equal the "calculated" or "expected" utilization of 6.5 and 7.5 Cal/gm, respectively. Nevertheless, as shown in Table 19, there were weekly periods when body weight gains of rats fed 1,3-butanediol or pelargonic acid equaled or exceeded the weight gains made on the 30% fat diet. However, it must be concluded that the 30% fat diet produced the most consistent results, followed closely by the 1,3-butanediol and pelargonic acid diets, respectively.

It may be possible that the caloric values of 6.5 Cal/gm for 1,3-butanediol and 7.5 Cal/gm for pelargonic acid are a little higher than the actual biological caloric densities. On the other hand, it is possible that these compounds are not as well utilized as corn oil. In this respect, it may be noted from the survival data given in Table 20 that the survival of the rats fed 1,3-butanediol was good. In contrast, the greatest mortality was observed in the animals fed pelargonic acid. Upon gross examination, however, no obvious abnormalities were noted in any of the internal organs.

The extent of urinary ketosis was found to be related to the time interval after feeding at which the samples were taken. Previously, urinary ketone bodies were estimated following the morning feeding. The results (Table 21) indicated that moderate ketosis may be present in animals fed the lower-protein 1,3-butanediol and pelargonic acid diets. At the conclusion of the experiment, it was decided to take serial urine samples for ketosis scores. As illustrated in Table 21, a peak urinary excretion of ketone bodies was observed at approximately 4 hours in animals fed 1,3-butanediol and possibly in the animals fed fat alone. It is possible that this peak ketonuria is related to the period of maximum absorption and metabolism of both compounds. If this is correct, then these data would tend to indicate that absorption of 1,3-butanediol is relatively slow. It must be remembered, however, that these data represent only an estimate of the extent of ketonuria, since methods for reliable and precise determinations of ketone bodies in blood and urine were not available. Nevertheless, the data reported here indicated that some consideration must be given to the interval of time following feeding when ketogenic diets and materials are evaluated.

Summary

An eight-week intubation experiment using equal volumes of isocaloric diets indicates that 1,3-butanediol and pelargonic acid

TABLE 19

AVERAGE BODY WEIGHT GAINS OF RATS INTUBATED WITH AN EQUAL VOLUME OF ISOCALORIC LIQUID DIETS CONTAINING CORN OIL ALONE AND WITH 1,3-BUTANEDIOL AND PELARGONIC ACID

AVERAGE WEIGHT GAINS FROM START TO FINISH OF EXPT. (GRAMS)

	1 Wk.	2 Wk.	3 Wk.	4 Wk.	5 Wk.	6 Wk.	7 Wk.	8 Wk.
30% Fat	15.7	38.0	54.8	72.6	96.0	97.3	106.4	127.8
30% Fat + 20% B.D.	8.5	30.5	45.2	59.3	81.6	78.1	92.5	111.3
30% Fat + 20% B.D. (H.P.)	8.5	32.9	49.3	65.5	83.2	78.5	94.5	114.1
30% Fat + 20% P.A.	2.0	26.0	42.1	53.6	76.1	73.3	83.3	103.1
30% Fat + 20% P.A. (H.P.)	9.3	30.5	43.9	69.6	74.4	73.0	82.3	95.5
Purina + Water	26.6	52.3	81.4	106.1	129.8	162.9	185.3	204.4

AVERAGE WEIGHT GAINS - ELIMINATING FIRST WEEK (GRAMS)

	1-4 Wk.	1-5 Wk.	1-6 Wk.	1-7 Wk.	1-8 Wk.
30% Fat	56.9	80.3	81.6	90.7	112.1
30% Fat + 20% B.D.	50.8	73.1	69.6	84.0	102.8
30% Fat + 20% B.D. (H.P.)	57.0	74.7	70.0	86.0	105.6
30% Fat + 20% P.A.	51.6	74.1	71.3	81.3	101.1
30% Fat + 20% P.A. (H.P.)	50.3	65.1	63.7	73.0	86.2
Purina + Water	83.5	107.2	140.3	162.7	181.8

AVERAGE WEEKLY WEIGHT GAINS (GRAMS)

	1 Wk.	2 Wk.	3 Wk.	4 Wk.	5 Wk.	6 Wk.	7 Wk.	8 Wk.
30% Fat	15.7	22.3	16.8	17.8	23.4	1.3	9.1	21.4
30% Fat + 20% B.D.	8.5	22.0	14.7	14.1	22.3	-3.5	14.4	16.8
30% Fat+20% B.D.(H.P.)	8.5	24.4	16.4	16.2	17.7	-4.7	16.0	19.6
30% Fat + 20% P.A.	2.0	24.0	16.1	11.5	22.5	-2.8	10.0	19.8
30% Fat + 20% P.A.(H.P.)	9.3	21.2	13.4	15.7	14.8	-1.4	9.3	13.2
Purina + Water	26.6	29.7	29.1	24.7	23.7	33.1	22.4	19.1

TABLE 20

AVERAGE WEEKLY SURVIVAL OF RATS*

	SURVIVAL TIME							
	1 Wk. %	2 Wk. %	3 Wk. %	4 Wk. %	5 Wk. %	6 Wk. %	7 Wk. %	8 Wk. %
30% Fat	90	90	90	90	90	90	60	50
30% Fat + 20% B.D.	100	100	100	100	100	100	100	90
30% Fat + 20% B.D. (H.P.)	100	90	90	90	90	90	80	80
30% Fat + 20% P.A.	90	70	70	60	60	60	60	30
30% Fat + 20% P.A. (H.P.)	100	90	80	70	70	70	60	50
Purina + Water	100	90	90	90	90	90	90	90

* All groups originally contained 10 animals per group. To convert to actual number of animals per group during any weekly period, divide the percentage value shown in the table by 10.

TABLE 21

URINARY KETOSIS SCORES OF RATS INTUBATED WITH LIQUID DIETS CONTAINING CORN OIL ALONE AND CORN OIL PLUS 1,3-BUTANEDIOL AND PELARGONIC ACID*

	AVERAGE RANGE OF SCORES OBTAINED 1 to 7 WEEKS**	8 WEEKS HOURS AFTER FEEDING			
		0***	2½	4½	6½
30% Fat	0-1	3	0	1	1
30% Fat + 20% B.D.	1-2	2	4	4	3.5
30% Fat + 20% B.D. (H.P.)	0-1	1	4	4	4
30% Fat + 20% P.A.	1-2	1	-	1	-
30% Fat + 20% P.A. (H.P.)	0-1	1	-	1	-
Purina + Water	0-1	0	-	0	-

* Urinary ketosis determined qualitatively using Ketostix.
** Usually determined after morning feeding.
*** Animals fasted 18 hours.

0 - No ketosis.
1 - Slight ketosis.
2 - Moderate ketosis
3 - Acute ketosis.
4 - Very severe ketosis.

approach but do not supply 6.5 and 7.5 Cal/gm, respectively. Utilization of both compounds for energy was demonstrated but complete utilization may have been affected by other factors such as rate of absorption. This was particularly true of pelargonic acid.

Urinary ketosis was found to be dependent upon the time after feeding during which urine samples were collected. This phenomenon may be related to the period of peak absorption and metabolism of fatty substances in the diet.

The data obtained fully support the original assumption that certain compounds, such as 1,3-butanediol and others, may exist and have promise as carbohydrate replacements in high-energy diets.

Experiment Seven
PROTEIN, FAT, AND 1,3-BUTANEDIOL INTERRELATIONSHIPS IN HIGH-ENERGY DIETS

Introduction

The results of previous experiments, particularly Experiment Six, indicated that high protein levels may have enhanced the utilization of fat or 1,3-butanediol. This is not a new concept. The importance of ample amounts of protein or amino acids for maximum energy utilization has often been discussed in the literature. For example, arctic inhabitants and explorers have been known to subsist on high-caloric diets principally composed of protein and fat. Since similar high-energy diets consisting of protein, fat and/or high-energy compounds may find application in space travel, it became desirable to examine more fully the possible interrelationships between protein, fat and 1,3-butanediol. In addition, the effects of a dietary supplement of emulsifiers and anti-ketogenic materials were also investigated.

The objectives of this long-term study were as follows:

1. To determine the interactions and effects of adequate and high-dietary levels of protein on the utilization by the rat of diets containing various levels of fat and 1,3-butanediol.

2. To determine the effect of soy lecithin (emulsifier), and calcium lactate and sodium propionate (anti-ketogenic materials) as supplements in high-energy diets.

Experimental Procedure

Fifteen equally-weighted groups of 10 animals each, a total of 150 rats, were used in the study. The animals were Caesarean-derived "specific pathogen free" male rats (Charles River Breeding

Laboratories, Wilmington, Massachusetts) which weighed approximately 145 gm each. More mature animals were used in this study than in many previous studies in order to avoid the odor and palatability problems associated with the use of younger animals. All animals were housed in individual wire-bottom cages in a temperature- and humidity-controlled animal room. Experimental groups were as follows:

1. 10% Fat + 20% Casein
2. 10% Fat + 40% Casein
3. 30% Fat + 20% Casein
4. 30% Fat + 40% Casein
5. 30% Fat + 20% Casein + 20% 1,3-Butanediol
6. 30% Fat + 40% Casein + 20% 1,3-Butanediol
7. 30% Fat + 40% Casein + 20% 1,3-Butanediol + 0.5% Lecithin
8. 30% Fat + 20% Casein + 30% 1,3-Butanediol
9. 30% Fat + 33% Casein + 30% 1,3-Butanediol
10. 50% Fat + 20% Casein
11. 50% Fat + 40% Casein
12. 60% Fat + 20% Casein
13. 60% Fat + 20% Casein + 0.5% Lecithin
14. 60% Fat + 20% Casein + 2.5% Calcium Lactate + 2.5% Sodium Propionate
15. 60% Fat + 33% Casein

The experimental variables in this study may be described as follows (all values are on a dry-diet basis):

Protein	20%, 33% and 40% casein, giving protein levels of 18%, 28%, and 36%, respectively
Fat	10 to 60%
1,3-Butanediol	20 to 30%
Calories	4.0 to 6.7 Cal/gm
Cal/gm Protein	36.8 to 11.1
Per cent of Calories	
Protein	11 to 36%
Fat	22 to 83%
1,3-Butanediol	22 to 36%
Carbohydrate	1 to 61%

The composition and characteristics of the diets are given in Tables 22 and 23. Since, in many respects, the composition of these rations was unusual (up to 60% fat and 30% butanediol), an extensive study was required to develop preparatory techniques and formulations having the proper characteristics; i.e., a firm gel-structure which could bind all components of the ration. The result was an agar-gel diet, containing corn oil and lard in a 1:3 ratio, which could bind large amounts of fat and butanediol in a semisolid gel. These diets and distilled water were fed ad libitum.

COMPOSITION OF DIETS USE

	1 10% Fat 20% Casein	2 10% Fat 40% Casein	3 30% Fat 20% Casein	4 30% Fat 40% Casein	5 30% Fat 20% Casein 20% B.D.	6 30% Fat 40% Casein 20% B.D.	7 30% Fat 40% Casei 20% B.D. 0.5% Leci
	gm	gm	gm	gm	gm	gm	gm
Casein	200	400	200	400	200	400	400
Sucrose	304	204	204	104	104	4	1
Dextrose	152	102	102	52	52	2	1
Dextrin	152	102	102	52	52	2	1
Corn Oil	25	25	75	75	75	75	75
Lard	75	75	225	225	225	225	225
1,3-Butanediol					200	200	200
Lecithin, Soy							5
Calcium Lactate							
Sodium Propionate							
Salt Mix W	40	40	40	40	40	40	40
Vitamin Mixture	10	10	10	10	10	10	10
Choline Chloride	2	2	2	2	2	2	2
Agar	40	40	40	40	40	40	40
Sub-Total	1000	1000	1000	1000	1000	1000	1000
Water	750	750	750	750	750	750	750
Grand Total	1750	1750	1750	1750	1750	1750	1750

B.D. - 1,3-Butanediol.
Lecithin - Refined Soy lecithin.
Sodium Propionate - Approx. 77.1% propionic acid.
Calcium Lactate - Approx. 58.4% lactic acid.

TABLE 22

POSITION OF DIETS USED TO STUDY PROTEIN, FAT AND 1,3-BUTANEDIOL INTERRELATIONSHIPS

6 30% Fat 10% Casein 20% B.D.	7 30% Fat 40% Casein 20% B.D. 0.5% Lecithin	8 30% Fat 20% Casein 30% B.D.	9 30% Fat 33% Casein 30% B.D.	10 50% Fat 20% Casein	11 50% Fat 40% Casein	12 60% Fat 20% Casein	13 60% Fat 20% Casein 0.5% Lecithin	14 60% Fat 20% Case 2.5% Lac 2.5% Pro
gm	gm	gm	gm	gm	gm	gm	gm	gm
400	400	200	308	200	400	200	200	200
4	1	54	-	104	4	54	51	29
2	1	27	-	52	2	27	26	15
2	1	27	-	52	2	27	26	14
75	75	75	75	125	125	150	150	150
225	225	225	225	375	375	450	450	450
200	200	300	300					
	5						5	
								25
								25
40	40	40	40	40	40	40	40	40
10	10	10	10	10	10	10	10	10
2	2	2	2	2	2	2	2	2
40	40	40	40	40	40	40	40	40
1000	1000	1000	1000	1000	1000	1000	1000	1000
750	750	750	750	750	750	750	750	750
1750	1750	1750	1750	1750	1750	1750	1750	1750

TABLE 22

STUDY PROTEIN, FAT AND 1,3-BUTANEDIOL
TERRELATIONSHIPS

8 30% Fat 0% Casein 30% B.D.	9 30% Fat 33% Casein 30% B.D.	10 50% Fat 20% Casein	11 50% Fat 40% Casein	12 60% Fat 20% Casein	13 60% Fat 20% Casein 0.5% Lecithin	14 60% Fat 20% Casein 2.5% Lactate 2.5% Propionate	15 60% Fat 33% Casein
gm	gm	gm	gm	gm	gm	gm	gm
200	308	200	400	200	200	200	308
54	-	104	4	54	51	29	-
27	-	52	2	27	26	15	-
27	-	52	2	27	26	14	-
75	75	125	125	150	150	150	150
225	225	375	375	450	450	450	450
300	300						
					5		
						25	
						25	
40	40	40	40	40	40	40	40
10	10	10	10	10	10	10	10
2	2	2	2	2	2	2	2
40	40	40	40	40	40	40	40
1000	1000	1000	1000	1000	1000	1000	1000
750	750	750	750	750	750	750	750
1750	1750	1750	1750	1750	1750	1750	1750

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CHARACTERISTICS OF DIETS USED TO ST
INTE

Diet	CALORIC CONTENT Cal./gm D.B.	Cal./gm W.B.	Cal./gm Protein	CALORIC DISTRIBUTION* Total calories as Protein %	Fat %	Carbo** %	B.D. %	Lec. %	Lac %
1. 10% Fat + 20% Casein	4.0	2.3	22.6	18	22	61	-	-	-
2. 10% Fat + 40% Casein	4.0	2.3	11.1	36	22	42	-	-	-
3. 30% Fat + 20% Casein	5.1	2.9	28.1	14	53	33	-	-	-
4. 30% Fat + 40% Casein	5.1	2.9	13.9	29	54	17	-	-	-
5. 30% Fat + 20% Casein + 20% B.D.	5.4	3.1	30.4	13	49	16	22	-	-
6. 30% Fat + 40% Casein + 20% B.D.	5.4	3.1	15.0	27	50	1	22	-	-
7. 30% Fat + 40% Casein + 20% B.D. + 0.5% Lec.	5.4	3.1	15.0	26	50	1	22	1	-
8. 30% Fat + 20% Casein + 30% B.D.	5.8	3.3	31.6	13	47	8	32	-	-
9. 30% Fat + 33% Casein + 30% B.D.	5.6	3.2	20.4	20	48	1	31	-	-
10. 50% Fat + 20% Casein	6.1	3.5	33.8	12	74	14	-	-	-
11. 50% Fat + 40% Casein	6.0	3.4	16.7	24	75	1	-	-	-
12. 60% Fat + 20% Casein	6.7	3.8	36.6	11	82	7	-	-	-
13. 60% Fat + 20% Casein + 0.5% Lec.	6.7	3.8	36.8	11	81	7	-	1	-
14. 60% Fat + 20% Casein + 2.5% Lact. + 2.5% Propn.	6.5	3.7	35.9	11	83	4	-	-	1
15. 60% Fat + 33% Casein	6.5	3.7	23.7	17	82	1	-	-	-

* Caloric calculation based on the following:
Protein- - - - - - - - - - - - -4 Cal./gm
Fat- - - - - - - - - - - - - - -9 Cal./gm
Carbohydrate- - - - - - - - - - 4 Cal./gm
1,3-Butanediol- - - - - - - - - 6 Cal./gm
Lecithin- - - - - - - - - - - - 9 Cal./gm
Calcium Lactate- - - - - - - - -2 Cal./gm
Sodium Propionate- - - - - - - -3 Cal./gm

** Includes calories from sucrose used as a diluent in the vitamin mix.

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TABLE 23

TICS OF DIETS USED TO STUDY PROTEIN, FAT AND 1,3-BUTANEDIOL INTERRELATIONSHIPS

CALORIC DISTRIBUTION*					DIET COMPOSITION											
calories as					Protein		Fat		B.D.		Carbo.		Lec.		Lactate	
Carbo**	B.D.	Lec.	Lact.	Propn.	D.B.	W.B.	D.B.	W.B.	D.B.	W.B.	D.B.	W.B.	D.B.	W.B.	D.B.	W.
%	%	%	%	%	%		%		%		%		%		%	
61	-	-	-	-	18.0	10.3	10.0	5.7	-	-	61.7	35.3	-	-	-	-
42	-	-	-	-	36.0	20.6	10.0	5.7	-	-	41.7	23.8	-	-	-	-
33	-	-	-	-	18.0	10.3	30.0	17.1	-	-	41.7	23.8	-	-	-	-
17	-	-	-	-	36.0	20.6	30.0	17.1	-	-	21.7	12.4	-	-	-	-
16	22	-	-	-	18.0	10.3	30.0	17.1	20.0	11.4	21.7	12.4	-	-	-	-
1	22	-	-	-	36.0	20.6	30.0	17.1	20.0	11.4	1.7	1.0	-	-	-	-
1	22	1	-	-	36.0	20.6	30.0	17.1	20.0	11.4	1.2	0.7	0.5	0.3	-	-
8	32	-	-	-	18.0	10.3	30.0	17.1	30.0	17.1	11.7	6.7	-	-	-	-
1	31	-	-	-	27.7	15.8	30.0	17.1	30.0	17.1	0.9	0.5	-	-	-	-
14	-	-	-	-	18.0	10.3	50.0	28.6	-	-	21.7	12.4	-	-	-	-
1	-	-	-	-	36.0	20.6	50.0	28.6	-	-	1.7	1.0	-	-	-	-
7	-	-	-	-	18.0	10.3	60.0	34.3	-	-	11.7	6.7	-	-	-	-
7	-	1	-	-	18.0	10.3	60.0	34.3	-	-	11.2	6.4	0.5	0.3	-	-
4	-	-	1	1	18.0	10.3	60.0	34.3	-	-	5.8	3.3	-	-	2.5	1.
1	-	-	-	-	27.7	15.8	60.0	34.3	-	-	0.9	0.5	-	-	-	-

D.B. - Dry basis, excluding added water in agar-gel diet preparation. S
W.B. - Wet basis, agar-gel diets as consumed.
Carbo. - Carbohydrate.
B.D. - 1,3-Butanediol.
Lec. - Lecithin, refined soy bean.
Lact. - Calcium lactate, approximately 58.4% lactic acid.
Propn. - Sodium propionate, approximately 77.1% propionic acid.

tamin mix.

OTEIN, FAT AND 1,3-BUTANEDIOL
IONSHIPS

DIET COMPOSITION														
	Protein		Fat		B.D.		Carbo.		Lec.		Lactate		Propionate	
Propn.	D.B.	W.B.	D.B.	W.B.	D.B.	W.B.	D.B.	W.B.	D.B.	W.B.	D.B.	W.B.	D.B.	W.B.
%	%		%		%		%		%		%		%	
-	18.0	10.3	10.0	5.7	-	-	61.7	35.3	-	-	-	-	-	-
-	36.0	20.6	10.0	5.7	-	-	41.7	23.8	-	-	-	-	-	-
-	18.0	10.3	30.0	17.1	-	-	41.7	23.8	-	-	-	-	-	-
-	36.0	20.6	30.0	17.1	-	-	21.7	12.4	-	-	-	-	-	-
-	18.0	10.3	30.0	17.1	20.0	11.4	21.7	12.4	-	-	-	-	-	-
-	36.0	20.6	30.0	17.1	20.0	11.4	1.7	1.0	-	-	-	-	-	-
-	36.0	20.6	30.0	17.1	20.0	11.4	1.2	0.7	0.5	0.3	-	-	-	-
-	18.0	10.3	30.0	17.1	30.0	17.1	11.7	6.7	-	-	-	-	-	-
-	27.7	15.8	30.0	17.1	30.0	17.1	0.9	0.5	-	-	-	-	-	-
-	18.0	10.3	50.0	28.6	-	-	21.7	12.4	-	-	-	-	-	-
-	36.0	20.6	50.0	28.6	-	-	1.7	1.0	-	-	-	-	-	-
-	18.0	10.3	60.0	34.3	-	-	11.7	6.7	-	-	-	-	-	-
-	18.0	10.3	60.0	34.3	-	-	11.2	6.4	0.5	0.3	-	-	-	-
1	18.0	10.3	60.0	34.3	-	-	5.8	3.3	-	-	2.5	1.4	2.5	1.4
-	27.7	15.8	60.0	34.3	-	-	0.9	0.5	-	-	-	-	-	-

D.B. - Dry basis, excluding added water in agar-gel diet preparation. See Table 22.
W.B. - Wet basis, agar-gel diets as consumed.
Carbo. - Carbohydrate.
B.D. - 1,3-Butanediol.
Lec. - Lecithin, refined soy bean.
Lact. - Calcium lactate, approximately 58.4% lactic acid.
Propn. - Sodium propionate, approximately 77.1% propionic acid.

TABLE 24

EFFECTS OF PROTEIN, FAT AND 1.3-BUTANEDIOL LEVELS ON 4-WEEK WEIGHT GAIN, NUTRIENT INTAKE, NUTRIENT EFFICIENCY AND URINARY KETOSIS

Diet	4-Week Body Weight Gain*	NUTRIENT INTAKE				NUTRIENT EFFICIENCY				URINARY KETOSIS***	
		Food**				Food					
		Wet	Dry	Protein	Calories	Wet	Dry	Protein	Calorie	Score	Rating
	gm	gm	gm	gm	Cal.	%	%				
1. 10% Fat + 20% Casein	159.7	786	448	81.0	1808	20.3	35.6	1.97	8.8	1.6	Slight
2. 10% Fat + 40% Casein	173.8	775	442	159.7	1783	22.4	39.3	1.09	9.7	1.6	Slight
3. 30% Fat + 20% Casein	179.2	700	399	72.1	2030	25.6	44.9	2.49	8.8	1.6	Slight
4. 30% Fat + 40% Casein	173.2	673	384	138.6	1952	25.7	45.1	1.25	8.9	2.2	Small
5. 30% Fat + 20% Casein + 20% B.D.	142.6	634	361	65.3	1965	22.5	39.5	2.18	7.3	3.0	Moderate
6. 30% Fat + 40% Casein + 20% B.D.	158.9	609	347	125.5	1888	26.1	45.8	1.27	8.4	1.2	Slight
7. 30% Fat + 40% Casein + 20% B.D. + 0.5% Lec.	148.1	562	320	115.8	1742	26.4	46.3	1.28	8.5	1.0	Slight
8. 30% Fat + 20% Casein + 30% B.D.	99.9	529	302	54.5	1746	18.9	33.1	1.83	5.7	3.2	Moderate
9. 30% Fat + 33% Casein + 30% B.D.	119.8	540	308	85.3	1728	22.2	38.9	1.40	6.9	2.4	Small
10. 50% Fat + 20% Casein	143.1	579	330	59.6	2027	24.7	43.4	2.40	7.1	2.0	Small
11. 50% Fat + 40% Casein	177.0	601	343	123.8	2043	29.5	51.6	1.43	8.7	2.0	Small
12. 60% Fat + 20% Casein	164.3	616	351	63.4	2341	26.7	46.8	2.59	7.0	0.4	None
13. 60% Fat + 20% Casein + 0.5% Lec.	142.7	549	313	56.5	2086	26.0	45.6	2.53	6.8	2.2	Small
14. 60% Fat + 20% Casein + 2.5% Lact. + 2.5% Propn.	154.9	562	320	57.9	2079	27.6	48.4	2.68	7.5	1.6	Slight
15. 60% Fat + 33% Casein	175.5	567	323	89.6	2098	31.0	54.3	1.96	8.4	1.0	Slight

B.D. - 1.3-Butanediol; Lec. - Lecithin; Lact. - Calcium Lactate; Propn. - Sodium Propionate.

* Butanediol groups penalized because first week body weight gain is included without any adjustment.
** Since diets were fed in the wet sugar-gel state, calculations were based on original wet-basis values.
*** Urinary Ketosis determined qualitatively with Ketostix.

Food Efficiency - grams body weight gain per 100 gm food consumed.
Protein Efficiency - grams body weight gain per one gm protein consumed.
Caloric Efficiency - grams body weight gain per 100 calories consumed.

TABLE 25

EFFECTS OF PROTEIN, FAT AND 1,3-BUTANEDIOL ON 8-WEEK WEIGHT GAIN, NUTRIENT INTAKE, AND NUTRIENT EFFICIENCY*

Diet	8-Week Body Weight Gain	NUTRIENT INTAKE				NUTRIENT EFFICIENCY			
		Food				Food			
		Wet	Dry	Protein	Cal.	Wet	Dry	Prot.	Cal.
	gm	gm	gm	gm	Cal.	%	%		Cal.
1. 10% Fat + 20% Casein	270	1575	898	162	3623	17	30	1.7	7.4
2. 10% Fat + 40% Casein	280	1557	888	321	3582	18	32	0.9	7.8
3. 30% Fat + 20% Casein	304	1463	834	151	4243	21	36	2.0	7.2
4. 30% Fat + 40% Casein	290	1400	789	288	4060	21	36	1.0	7.1
5. 30% Fat + 20% Casein + 20% B.D.	257	1326	755	137	4110	19	34	1.9	6.3
6. 30% Fat + 40% Casein + 20% B.D.	265	1286	733	265	2987	21	36	1.0	6.6
7. 30% Fat + 40% Casein + 20% B.D.+ 0.5% Lec.	247	1196	681	246	3707	21	36	1.0	6.7
8. 30% Fat + 20% Casein + 30% B.D.	206	1176	671	121	3881	18	31	1.7	5.3
9. 30% Fat + 33% Casein + 30% B.D.	224	1209	689	191	3869	18	32	1.2	5.8
10. 50% Fat + 20% Casein	255	1247	711	128	4365	20	36	2.0	5.8
11. 50% Fat + 40% Casein	280	1258	717	259	4277	22	39	1.1	6.5
12. 60% Fat + 20% Casein	287	1274	726	131	4841	22	40	2.2	5.9
13. 60% Fat + 20% Casein + 0.5% Lec.	259	1165	664	120	4427	22	39	2.2	5.9
14. 60% Fat + 20% Casein + 2.5% Lact. + 2.5% Propn.	260	1183	674	122	4377	22	39	2.1	5.9
15. 60% Fat + 33% Casein	289	1191	679	188	4407	24	42	1.5	6.5

B.D. - 1,3-Butanediol; Lec. - Lecithin; Lact. - Calcium Lactate; Propn. - Sodium Propionate.

* Since diets were fed in the wet agar-gel state, calculations were based on original wet-basis values.

Cal. Effic. - gm body wt. gain/100 Cal.
Prot. Effic. - gm body wt. gain/one gm prot. consumed
Food efficiency - gm body wt. gain/100 gm food consumed.

TABLE 26

EFFECTS OF PROTEIN, FAT, AND 1,3-BUTANEDIOL ON 30-WEEK WEIGHT GAIN, NUTRIENT INTAKE, NUTRIENT EFFICIENCY AND SURVIVAL*

Diet	No. Rats	30-Week Body Weight Gain	NUTRIENT INTAKE				NUTRIENT EFFICIENCY			
			Food				Food			
			Wet	Dry	Prot.	Cal.	Wet	Dry	Prot.	Cal.
		gm	gm	gm	gm	Cal.	%	%		Cal.
1. 10% Fat + 20% Casein	9	445±20**	5932	3381	609	13644	7.5	13.2	0.73	3.3
2. 10% Fat + 40% Casein	10	448±13	5762	3284	1182	13253	7.8	13.6	0.38	3.4
3. 30% Fat + 20% Casein	10	518±35	5470	3118	561	15863	9.5	16.6	0.92	3.3
4. 30% Fat + 40% Casein	8	509±40	5437	3099	1116	15767	9.4	16.4	0.46	3.2
5. 30% Fat + 20% Casein + 20% B.D.	9	491±20	5110	2913	524	15841	9.6	16.9	0.94	3.1
6. 30% Fat + 40% Casein + 20% B.D.	9	447±39	5029	2867	1032	15589	8.9	15.6	0.43	2.9
7. 30% Fat + 40% Casein + 20% B.D. + .5% Lec.	7	440±34	4705	2682	966	14586	9.4	16.4	0.46	3.0
8. 30% Fat + 20% Casein + 30% B.D.	9	397±37	4627	2637	475	15269	8.6	15.1	0.84	2.6
9. 30% Fat + 33% Casein + 30% B.D.	8	338±60	4602	2623	734	14726	7.3	12.9	0.46	2.3
10. 50% Fat + 20% Casein	9	484±40	4885	2784	501	17098	9.9	17.4	0.97	2.8
11. 50% Fat + 40% Casein	7	482±18	4853	2766	996	16500	9.9	17.4	0.48	2.9
12. 60% Fat + 20% Casein	10	553±42	5003	2852	513	19011	11.1	19.4	1.08	2.9
13. 60% Fat + 20% Casein + .5% Lec.	9	524±29	4759	2713	488	18084	11.0	19.3	1.07	2.9
14. 60% Fat + 20% Casein + 2.5% Lact. + 2.5% Propn.	7	432±30	4702	2680	482	17397	9.2	16.1	0.90	2.5
15. 60% Fat + 33% Casein	8	564±34	4769	2718	761	17645	11.8	20.8	0.74	3.2

B.D. - 1,3-Butanediol; Lec. - Lecithin; Lact. - Calcium Lactate; Propn. - Sodium Propionate.

*Since diets were fed in the wet-agar gel state, calculations were based on original wet-basis values.

**Standard error.

Food Efficiency - gm body wt. gain/100 gm food consumed.
Protein Effic. - gm body wt. gain/1 gm protein consumed.
Cal. Effic. - gm body wt. gain/100 cal. consumed.

TABLE 27

CUMULATIVE AND WEEKLY BODY WEIGHT GAINS OF MALE RATS FED VARIOUS LEVELS OF PROTEIN, FAT AND 1,3-BUTANEDIOL

Group	WEEK 1		2		3		4		5		6		7		8	
	Cum. gm	Wk. gm	Cum. gm	Wk. gm	Cum. gm	Wk. gm	Cum. gm	Wk. gm	Cum. gm	Wk. gm	Cum. gm	Wk. gm	Cum. gm	Wk. gm	Cum. gm	Wk. gm
1. 10% Fat + 20% Casein	13.5	13.5	82.3	68.8	132.1	49.8	159.7	27.6	194.0	34.3	215.4	21.4	248.1	32.7	269.6	21.5
2. 10% Fat + 40% Casein	22.8	22.8	85.8	63.0	138.5	52.7	173.8	35.3	206.1	32.3	226.1	20.0	259.8	33.7	280.3	20.5
3. 30% Fat + 20% Casein	24.2	24.2	88.9	64.7	141.8	52.9	179.2	37.4	212.8	33.6	245.7	32.9	279.2	33.5	303.6	24.4
4. 30% Fat + 40% Casein	25.3	25.3	88.8	63.5	136.2	47.4	173.2	37.0	203.0	29.8	230.1	27.1	264.2	34.1	289.6	25.4
5. 30% Fat + 20% B.D. + 20% Casein	13.1	13.1	74.0	60.9	113.8	39.8	142.6	28.8	169.4	26.8	197.3	27.9	236.3	39.0	256.9	20.6
6. 30% Fat + 20% B.D. + 40% Casein	29.8	29.8	83.9	54.1	124.4	40.5	158.9	34.5	185.7	26.8	213.1	27.4	235.1	22.0	265.1	30.0
7. 30% Fat + 20% B.D.+ 40% Casein + 0.5% Lec.	27.8	27.8	76.8	49.0	118.0	41.0	148.1	30.1	178.7	30.6	200.9	22.2	235.2	34.3	247.4	12.2
8. 30% Fat + 30% B.D.+ 20% Casein	8.5	8.5	49.5	41.0	81.1	31.6	99.9	18.8	119.0	19.1	150.7	31.7	185.9	35.2	205.5	19.6
9. 30% Fat + 30% B.D.+ 33% Casein	17.3	17.3	59.1	41.8	97.7	38.6	119.8	22.1	139.4	19.6	168.3	28.9	202.7	34.4	224.1	21.4
10. 50% Fat + 20% Casein	23.9	23.9	72.3	48.4	115.3	43.0	143.1	27.8	163.8	20.7	197.4	33.6	232.2	34.8	255.1	22.9
11. 50% Fat + 40% Casein	39.0	39.0	90.7	51.7	140.5	49.8	177.0	36.5	197.4	20.4	221.4	24.0	253.6	32.2	280.1	26.5
12. 60% Fat + 20% Casein	34.6	34.6	80.1	45.5	132.2	52.1	164.3	32.1	198.3	34.0	225.3	27.0	257.3	32.0	286.9	29.6
13. 60% Fat + 20% Casein + 0.5% Lec.	34.3	34.3	78.4	44.1	112.2	33.8	142.7	30.5	167.9	25.2	192.6	24.7	227.8	35.2	259.3	31.5
14. 60% Fat + 20% Casein + 2.5% Lact. + 2.5% Propn.	24.7	24.7	79,6	54.9	119.6	40.0	154.9	35.3	178.5	23.6	205.1	26.6	235.9	30.8	260.4	24.5
15. 60% Fat + 33% Casein	35.7	35.7	92.9	57.2	142.2	49.3	175.5	33.3	187.8	12.3	229.6	41.8	265.1	35.5	288.6	23.5

TABLE 28

CUMULATIVE BI WEEKLY AVERAGE BODY WEIGHT GAINS OF MALE RATS FED VARIOUS LEVELS OF PROTEIN, FAT, AND 1,3-BUTANEDIOL

	8 Wk.	10 Wk.	12 Wk.	14 Wk.	16 Wk.	18 Wk.	20 Wk.	22 Wk.	24 Wk.	26 Wk.	28 Wk.	30 Wk.
	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm
1. 10% Fat + 20% Casein	270	307	333	373	392	412	422	439	437	444	463	445
2. 10% Fat + 40% Casein	280	316	352	382	400	415	435	437	425	480	454	448
3. 30% Fat + 20% Casein	304	353	387	433	456	489	509	502	500	524	538	518
4. 30% Fat + 40% Casein	290	329	362	396	420	457	460	479	462	472	504	509
5. 30% Fat + 20% Casein + 20% B.D.	257	301	336	373	409	419	433	478	452	474	499	491
6. 30% Fat + 40% Casein + 20% B.D.	265	295	324	356	370	418	430	449	440	456	471	447
7. 30% Fat + 40% Casein + 20% B.D. + 0.5% Lec	247	280	308	332	358	368	369	386	386	408	444	440
8. 30% Fat + 20% Casein + 30% B.D.	206	226	265	289	303	334	339	361	372	391	410	397
9. 30% Fat + 33% Casein + 30% B.D.	224	253	272	307	308	325	331	347	342	340	343	338
10. 50% Fat + 20% Casein	255	303	340	377	417	443	443	451	455	476	491	484
11. 50% Fat + 40% Casein	280	307	344	377	402	427	433	444	442	454	465	482
12. 60% Fat + 20% Casein	287	338	387	425	467	478	497	518	524	546	576	553
13. 60% Fat + 20% Casein	259	282	311	337	369	395	404	429	437	466	484	524
14. 60% Fat + 20% Casein + 2.5% Lact + 2.5% Propn	260	288	320	348	368	389	384	397	389	405	411	432
15. 60% Fat + 33% Casein	289	324	355	385	403	431	443	464	456	467	522	564

B.D. - 1,3-Butanediol; Lec - lecithin; Lact - Calcium Lactate; Propn - Sodium Propionate.

Results

The data obtained have been evaluated in terms of body weight gain, nutrient intake, food efficiency, protein efficiency, caloric efficiency, degree of ketosis, survival, internal organ weights, serum enzyme biochemistry, and microscopic periodontal tissue changes. Diet 1 (10% fat + 20% casein) served as the regular protein level control, while diet 2 (10% fat + 40% casein) was the high protein control.

The results of the feeding studies have been divided into 3 growth periods, 4, 8, and 30 weeks, and are presented in Tables 24, 25, and 26, respectively.

Body Weight Gain

At best, the interpretation of growth and body weight differences as indicated by body weight gain is difficult. In this experiment, the body weight gain changes with age were evaluated continuously, but particularly after 4, 8 and 30 weeks on test. Cumulative and weekly average body weight gains of the animals from 1 to 8 weeks on test are given in Table 27. Bi-weekly cumulative average weight gains from 8 to 30 weeks on test are shown in Table 28.

Throughout the test, there were differences in weight gain among the various treatment groups, but there were no "unthrifty" animals. All of the rats appeared to be in good health at all times.

Examination of the 4-week weight gains reveals that groups which were fed diets containing 30% 1,3-butanediol gained the least weight. The weight gains were improved, however, when dietary protein was raised from 18 to 28%. The weight gains of the 20% 1,3-butanediol groups were in the intermediate range. At 4 weeks, there was a general trend toward greater weight gains with the high protein levels. For example, 4 out of 5 diets supporting the highest body weight increases (173 gm or more) contained either 33% or 40% casein rather than the low level of 20% (Table 24). It is also interesting to note that 10, 30, 50, and 60% fat diets are represented in the top five diets for body weight gain, and with the exception of the two groups which received diets containing high levels of casein, weight gains of the 1,3-butanediol-fed animals were in the lowest bracket.

The adaptation period observed in earlier investigations was also demonstrated in this as illustrated in Table 27; animals fed 20% butanediol and 20% protein (diet 5) gained only 13.1 gm in the first week of the study. However, during subsequent weeks,

the weight gain by this group approximated that of the control animals. When the protein level was increased to 40% (diet 6), these animals grew at a rate similar to those of the controls, indicating the beneficial effects of high protein diets in reducing the adaptation period.

After 8 weeks on test, the weight gain trends described above for 4 weeks were maintained with the exception that high protein levels were no longer significantly better.

The 30-week weight gains were also lowest in the 2 groups fed 30% 1,3-butanediol and highest on the 60% fat diets. However, the 3 groups of rats fed 20% 1,3-butanediol had weight gains equivalent to those of the 2 control diets. As with the 8-week weight gains, the 30-week weight gains did not appear to be influenced by the level of protein in the diet. A comparison of 27 statistically significant 30-week body weight gain differences is given in Table 29.

Nutrient Intake

Since food consumption in the rat is largely influenced by caloric intake, the caloric consumption figures given in Tables 24, 25, and 26 probably provide the most reliable way to compare nutrient intakes on the various diets.

At 4 weeks, as may be expected because of a higher caloric content, animals fed diets containing 1,3-butanediol consumed less food than comparable animals fed diets containing the same amount of fat (30% fat). In addition, there was a reduction in caloric intake in 1,3-butanediol-containing diets 7, 8, and 9; diets 8 and 9 contained 30% 1,3-butanediol.

The 8- and 30-week nutrient intakes continued to follow, with some improvement, the general pattern set at 4 weeks. While less feed was consumed by the rats which were fed 20% and 30% levels of 1,3-butanediol than by those fed on the 10% fat control or by groups fed on 30% fat, the animals receiving 1,3-butanediol had caloric intakes higher than those of the controls. Furthermore, the 20% 1,3-butanediol animals' caloric intakes approached those of the 30% fat groups. The highest caloric consumption occurred in groups receiving 60% fat. Protein intakes varied in accordance with levels in the diets.

Food Efficiency

As compared to the controls, good food efficiencies were generally obtained with all test groups and at all ages.

TABLE 29

COMPARISON OF SIGNIFICANT 30-WEEK WEIGHT GAIN DIFFERENCES IN RATS FED VARIOUS LEVELS OF PROTEIN, FAT, AND 1,3-BUTANEDIOL*

Groups Compared	Difference	Significance Level
	gm	
10% Fat + 20% Casein vs 60% Fat + 20% Casein **	108	5%
10% Fat + 20% Casein vs 60% Fat + 33% Casein **	119	5%
10% Fat + 20% Casein** vs 30% Fat + 33% Casein + 30% B.D.	107	5%
10% Fat + 40% Casein vs 60% Fat + 20% Casein **	105	5%
10% Fat + 40% Casein** vs 30% Fat + 33% Casein + 30% B.D.	110	5%
10% Fat + 40% Casein vs 60% Fat + 33% Casein **	116	5%
30% Fat + 20% Casein** vs 30% Fat + 20% Casein + 30% B.D.	121	5%
30% Fat + 20% Casein** vs 30% Fat + 33% Casein + 30% B.D.	180	1%
30% Fat + 40% Casein** vs 30% Fat + 20% Casein + 30% B.D.	112	5%
30% Fat + 40% Casein** vs 30% Fat + 33% Casein + 30% B.D.	171	1%
30% Fat + 20% Casein + 20% B.D.** vs 30% Fat + 33% Casein + 30% B.D.	153	1%
30% Fat + 40% Casein + 20% B.D. vs 60% Fat + 20% Casein**	106	5%
30% Fat + 40% Casein + 20% B.D. vs 60% Fat + 33% Casein**	117	5%
30% Fat + 40% Casein + 20% B.D.** vs 30% Fat + 33% Casein + 30% B.D.	109	5%
30% Fat + 40% Casein + 20% B.D. +0.5% Lec. vs 60% Fat + 20% Casein**	113	5%
30% Fat + 40% Casein + 20% B.D. +0.5% Lec. vs 60% Fat + 33% Casein **	124	5%
30% Fat + 40% Casein + 20% B.D. +0.5% Lec.** vs 30% Fat + 33% Casein + 30% B.D.	102	5%
30% Fat + 20% Casein + 30% B.D. vs 60% Fat + 20% Casein **	156	1%
30% Fat + 20% Casein + 30% B.D. vs 60% Fat + 20% Casein +0.5% Lec. **	127	1%
30% Fat + 20% Casein + 30% B.D. vs 60% Fat + 33% Casein **	167	1%
30% Fat + 33% Casein + 30% B.D. vs 50% Fat + 20% Casein **	146	1%
30% Fat + 33% Casein + 30% B.D. vs 50% Fat + 40% Casein **	144	1%
30% Fat + 33% Casein + 30% B.D. vs 60% Fat + 20% Casein **	215	1%
30% Fat + 33% Casein + 30% B.D. vs 60% Fat + 20% Casein +0.5% Lec. **	186	1%
30% Fat + 33% Casein + 30% B.D. vs 60% Fat + 33% Casein **	226	1%
60% Fat + 20% Casein** vs 60% Fat + 20% Casein + 2.5% Lact. + 2.5% Propn.	121	5%
60% Fat + 20% Casein + 2.5% Lact. + 2.5% Propn. vs 60% Fat + 33% Casein **	132	1%

* LSD at 5% Level = 95.3 gm; LSD at 1% Level = 124.9 gm.
** Is the group with the higher weight gain.

Excellent 4-week food efficiencies were obtained with the 50% and 60% fat diets. At these high levels of fat, the groups fed the higher protein diets demonstrated the best food efficiencies (diets 11 and 15). Moreover, high-protein levels appeared to increase the food efficiency of 1,3-butanediol-containing diets 6 and 7.

At 8 and at 30 weeks, the 20% 1,3-butanediol groups were superior to the controls in food efficiency. The 30% 1,3-butanediol animals had food efficiencies which almost equalled those of the controls. The best food efficiencies were observed in animals fed the 60% fat diets.

Protein Efficiency

Since the protein efficiency of diets containing more protein than that required by an animal cannot be of a high order, it is not unexpected that the best protein efficiencies were obtained with the diets containing the lower (20%) level of casein. This level of casein in the diet is considered to be more nearly optimal for the rat.

It was noted that at 4 weeks, the 3 highest protein efficiencies resulted from diets which also contained 60% fat. This was also true of the 8-week protein efficiencies. The inclusion of 1,3-butanediol in diets did not appear to impair protein efficiency at any age. In fact, when diets containing equal levels of protein are compared, protein efficiency at 30 weeks is better in all experimental diets, including those containing 1,3-butanediol, than in the controls.

Caloric Efficiency

As may be expected, diets containing the lower levels of fat generally gave the best caloric efficiencies. On the other hand, the diets containing 30% 1,3-butanediol were least efficient in caloric conversion.

The 4-week caloric efficiency values were highest in groups fed 10% and 30% fat plus 40% casein (diets 2 and 4). At 4 and 8 weeks, respectively, there was a trend toward better caloric efficiency with diets containing the higher level of casein. At the 8 and 30 week, the caloric efficiencies of animals fed 20% 1,3-butanediol were almost equal to those of the 30% fat groups. Furthermore, the caloric efficiency values of the 20% 1,3-butanediol animals, at 8 and 30 weeks, were generally equal to or surpassed those of animals fed the 50% and 60% fat diets.

Effect of Supplements

As mentioned previously, lecithin was added to some of the diets in order to test for a possible beneficial effect on 1,3-butanediol and fat absorption. Calcium lactate and sodium propionate were tested for an anti-ketogenic effect. Evaluation of these supplements at 4, 8, and 30 weeks revealed no consistent beneficial effects.

Urinary Ketone Bodies, Protein, Glucose and pH

Qualitative urinary ketosis measurements for 4 and 21 weeks on test are given in Tables 24 and 30, respectively. In addition, Table 30 shows the results of qualitative measurements at 21 weeks on test of urinary protein, glucose and pH. There were no unusual differences noted among the various groups.

Survival

At the termination of the experiment after 30 weeks, survival was 70 to 100%. Thus, out of an original 10 rats per group, no more than 3 animals died in any experimental group. This mortality did not appear to be related to the type of diet. Thirty-week survival figures are given in Table 26.

Organ Weights

The recording of organ weights at the termination of an experiment is desirable when there is reason to suspect that a toxic compound or factor may be present in the diet. In this experiment, it was of interest to determine if either the 1,3-butanediol or the other lipids present in the diets would alter liver or kidney weights. As shown in Table 31, there were no significant differences among any of the liver or kidney values when compared on a percentage of body weight basis.

Microscopic Periodontal Tissue Examination

Since the design of this experiment provided an opportunity to study the effects of various levels of fat, protein, and carbohydrate on dental health, Dr. Irving Glickman of Tufts University was contacted and expressed an interest in undertaking oral pathology studies. Accordingly, the heads from the 30-week animals were turned over to him with the agreement that periodic reports of the dental studies would be issued for quarterly and technical reports.

An abstract of the progress to date has been prepared by Dr. Glickman. It must be emphasized that only preliminary results

TABLE 30

QUALITATIVE MEASUREMENTS OF URINARY KETONE BODIES, PROTEIN, GLUCOSE, AND pH (21-22 WEEKS ON TEST)*

Diet	Ketone Bodies**	Protein***	Glucose****	pH
1. 10% Fat + 20% Casein	0.8	2.8	0.0	6.3
2. 10% Fat + 40% Casein	0.5	2.7	0.0	5.7
3. 30% Fat + 20% Casein	0.9	3.3	0.0	6.0
4. 30% Fat + 40% Casein	0.8	3.3	0.0	6.0
5. 30% Fat + 20% Casein + 20% B.D.	1.2	3.0	0.0	6.0
6. 30% Fat + 40% Casein + 20% B.D.	0.8	4.2	0.0	6.2
7. 30% Fat + 40% Casein + 20% B.D. + 0.5% Lec.	1.1	2.8	0.0	6.2
8. 30% Fat + 20% Casein + 30% B.D.	1.1	2.7	0.0	6.0
9. 30% Fat + 33% Casein + 30% B.D.	1.4	3.0	0.0	5.8
10. 50% Fat + 20% Casein	1.4	2.9	0.0	5.7
11. 50% Fat + 40% Casein	1.0	3.0	0.0	5.7
12. 60% Fat + 20% Casein	1.3	3.4	0.0	6.0
13. 60% Fat + 20% Casein + 0.5% Lec.	1.3	3.4	0.0	5.3
14. 60% Fat + 20% Casein + 2.5% Lact. + 2.5% Propn.	1.7	3.5	0.0	6.0
15. 60% Fat + 33% Casein	1.1	3.1	0.1	5.9

B.D. - 1,3-Butanediol; Lec. - Lecithin; Lact. - Calcium Lactate; Propn. - Sodium Propionate.

* Tests made with Ketostix and Combistix on overnight fasted animals.

** Ketone bodies: average of 3 determinations on Groups 1-11.
average of 2 determinations on Groups 12-15.
0-none; 1-small; 2-moderate; 3-large.

*** Protein: Mg. protein per 100 ml. of urine.
0-none; 1-trace; 2-30 mg.; 3-100 mg; 4-300 mg.; 5-over 1000 mg.

**** Glucose: 0-negative; 1-light; 2-medium; 3-heavy.

TABLE 31

WEIGHTS OF LIVERS AND KIDNEYS OF ANIMALS FED DIETS CONTAINING VARIOUS LEVELS OF PROTEIN, FAT AND 1,3-BUTANEDIOL*

Group	Liver % Body Weight	Kidney % Body Weight
1. 10% Fat + 20% Casein	2.05 ± 0.07**	0.55 ± 0.02
2. 10% Fat + 40% Casein	2.19 ± 0.07	0.53 ± 0.02
3. 30% Fat + 20% Casein	1.98 ± 0.07	0.47 ± 0.02
4. 30% Fat + 40% Casein	2.10 ± 0.07	0.50 ± 0.02
5. 30% Fat + 20% Casein + 20% B.D.	2.30 ± 0.06	0.52 ± 0.02
6. 30% Fat + 40% Casein + 20% B.D.	2.20 ± 0.05	0.59 ± 0.03
7. 30% Fat + 40% Casein + 20% B.D. + .5% Lec.	2.20 ± 0.02	0.56 ± 0.01
8. 30% Fat + 20% Casein + 30% B.D.	2.40 ± 0.08	0.54 ± 0.01
9. 30% Fat + 33% Casein + 30% B.D.	2.20 ± 0.14	0.63 ± 0.05
10. 50% Fat + 20% Casein	2.10 ± 0.13	0.49 ± 0.02
11. 50% Fat + 40% Casein	2.20 ± 0.10	0.48 ± 0.01
12. 60% Fat + 20% Casein	2.03 ± 0.05	0.49 ± 0.02
13. 60% Fat + 20% Casein + .5% Lec.	2.00 ± 0.04	0.48 ± 0.04
14. 60% Fat + 20% Casein + 2.5% Lact. + 2.5% Propn.	2.00 ± 0.09	0.57 ± 0.03
15. 60% Fat + 33% Casein	2.10 ± 0.07	0.52 ± 0.02

* No significant differences exist among any of the liver or kidney values.

** Indicates Standard Error.

have been obtained. However, these dental studies shall receive careful future evaluation as they promise to be highly interesting and informative.

The summary received from Dr. Glickman is presented below:

"Periodontal Tissue Changes Induced by 1,3-Butanediol in the Diet of Albino Rats. IRVING GLICKMAN, Tufts University School of Dental Medicine, Boston 11, Mass.

The effects of 1,3-butanediol in the diet of albino rats were studied microscopically in the periodontal tissues. 150 Charles River C.D. male albino rats weighing 100 grams were divided into 15 groups and fed diets of equal weight and consistency. Two groups served as controls. In 13 experimental groups the diet was altered by varied increases in the percentage of fat content or by adding comparable amounts of 1,3-butanediol or both. After 30 weeks the animals were sacrificed, frozen immediately at -40°F, and subsequently defrosted, fixed in neutral buffered formalin. Sections of the jaws of some of the groups have been decalcified and stained. Preliminary observation suggests the occurrence of fibrinoid changes and hyalinization of the principal fibers of the periodontal membrane and increased osteoclasis of the alveolar bone in the butanediol-treated animals which were not observed in animals on control or fat-supplemented diets."

Summary

Data have been collected on the feeding of high-energy diets (5.1 to 6.5 Cal/gm), composed principally of fat and protein with and without supplements of 20% and 30% 1,3-butanediol, to rats for a period of 30 weeks.

The results indicate that the rat has a remarkable ability to adjust to and thrive on high-calorie diets containing 30% to 60% fat, 18% to 36% protein, 20% 1,3-butanediol and very little carbohydrate. Overall utilization of the diets was most efficient with the 60% fat diets. Utilization was markedly impaired only when 1,3-butanediol was fed at the 30% level. High-protein levels had a beneficial effect in animals fed high-fat diets or 1,3-butanediol only during the first 4 weeks on test. While 20% 1,3-butanediol appeared to be normally metabolized, the preliminary results indicating that undesirable microscopic tissue changes may occur in animals fed 1,3-butanediol require careful continued study.

The data suggests that high-energy diets, in which almost all of the carbohydrate is replaced by fat or a combination of fat and 1,3-butanediol or some other high-energy metabolite, may show promise as for use in space travel.

Experiment Eight
INVESTIGATION OF SOME GLYCOLS AS POTENTIAL FEEDING COMPOUNDS

Introduction and Purpose

Since experimental work with 1,3-butanediol has shown that it is relatively nontoxic and can be utilized for energy to the extent of about 6 Cal/gm, an effort was made to learn more about members of the glycol and butanediol family. In view of the fact that published toxicity values have not always proved to be reliable, a test was conducted with corn oil (control), 1,3-butanediol, 1,4-butanediol anhydrous, 1,4-butanediol, 1,2,4-butanetriol and butynediol diacetate.

Experimental Procedure

A single large dose of each compound, corn oil (control), 1,3-butanediol, 1,4-butanediol anhydrous, 1,4-butanediol, 1,2,4-butanetriol, and butynediol diacetate, of 20 gm/kg, was orally administered to fasted rats which weighed about 200 gm each. Since an oral LD_{50} of 15 gm/kg is considered to be relatively harmless, it was decided to utilize a test dose of 20 gm/kg to provide a severe test.

Results and Summary

Experimental and literature values are compared in Table 32. The experimental results and literature tabulation agree on the low order of toxicity of 1,3-butanediol. On the other hand, the other butane glycols listed appear to be too toxic for dietary use. As for the glycols, in general, propylene glycol, having a heat of combustion of about 5 Cal/gm, is probably the least toxic.

III. Summary

During the period covered by this report, a total of 8 animal tests were completed. Three of these tests were concerned with the M.I.T. bio-assay for estimating the caloric density of dietary components. One test involved the investigation of the toxicity of a number of glycols. Four tests were carried out to study factors affecting the utilization and metabolism of "high-energy metabolites" in the form of fat, nonanoic (pelargonic) acid, and 1,3-butanediol.

TABLE 32

COMPARATIVE TOXICITY OF SOME GLYCOLS

Compound	EXPERIMENTAL: Rat (Approx. 200 gm) Oral Adm. 20 gm/kg.	LITERATURE: Rat LD_{50} gm/kg.	LITERATURE: Mouse LD_{50} ml./kg. ***
Corn Oil (control)	100% survival	----	----
1,3-Butanediol	100% survival (LD_{50} higher than 20 gm/kg.	18.6*	23.3
1,4-Butanediol (anhyd.)	Death in ½ hr.	----	----
1,4-Butanediol	Death in ½ hr.	----	2.1
1,2,4-Butanetriol	Death in 24 Hrs.	----	----
Butynediol diacetate	Death in 24 hrs.	----	----
2,3-Butanediol	----	----	9.0
Ethylene glycol	----	8.5*	13.8
Propylene glycol	----	26.4 *	30.8
Trimethylene glycol	----	16.0**	6.0

* Smyth, H.F., J. Eaton, and L. Fischer, J. Indust. Hyg. 23:259 (1941).
** vanWinkle, W., J. Pharm. 72:227 (1941).
*** Fischer, L., R. Kopf., A. Loeser, and G. Meyer, Z. ges. exp. Med. 115:22 (1949).

As a result of these tests, more information has been obtained on the control of factors which influence the reliability of the M.I.T. caloric bio-assay; these include variation caused by strain, initial body weight, and group weight range differences.

Biochemical metabolic studies on the comparative utilization and metabolism of octanoic acid (even-carbon, C_8) and nonanoic acid (odd-carbon, C_9) in high-fat diets supported the contention that odd-carbon fatty acids may be partly glucogenic and that more suitable odd-carbon compounds than nonanoic acid may prove capable of replacing a large portion of dietary carbohydrate.

Paired-feeding and intubation studies, which circumvented diet acceptance and palatability problems, indicated that nonanoic acid (pelargonic acid) and 1,3-butanediol were utilized for energy at approximately 6.0 Cal/gm. Of the two compounds, 1,3-butanediol was found to be more acceptable for inclusion in the ration.

A long-term (30-week) feeding study demonstrated that rats can utilize high-energy diets containing 18% protein, 30% to 60% fat, 30% fat plus 20% 1,3-butanediol, and very little carbohydrate. These data suggest the potential usefulness of 1,3-butanediol in similar formulations for astronaut rations.

SECTION D. SUMMARY

1. The synthesis of 2,4-dimethylheptanoic acid and the separation of its diastereoisomers has been completed. The purity of the compound has been verified by gas chromatography and infrared spectroscopy. Synthesis of the labeled acid by means of a malonic ester condensation is in progress.

2. The acute toxicity of 2,4-dimethylheptanoic acid has been determined. The oral LD_{50}(7 days) was estimated to be approximately 5 gm/kg. This slight toxicity is similar to those observed upon examination of other short-chain fatty acids and is not expected to be significant when the compound is included as part of a mixed diet.

3. Since it is expected that the unoxidized portion of ingested 2,4-dimethylheptanoic acid will consist of low molecular weight monocarboxylic and dicarboxylic acids, an analytical scheme has been developed for separation and identification of such compounds.

4. Further investigations of the factors influencing the M.I.T. caloric bio-assay indicate that initial body weight and weight spread within groups were most influential in maintaining reliability. Initial weights of 45-46 grams and weight spreads of 5-6 grams gave best results.

5. Biochemical, metabolic, and feeding tests supported the contention that odd-carbon fatty acids, e.g., nonanoic acid, are partly glucogenic even when included as part of a high-fat diet. Nonanoic acid, however, was found to be relatively poorly utilized by the rat. Other odd-carbon fatty acids may have some value, however.

6. Paired-feeding and intubation studies indicated that the relatively poor growth of animals fed 1,3-butanediol was a function of decreased food intake and not due to toxic or metabolic dysfunction.

7. A long term (30-week) feeding study demonstrated that the rat can utilize 1,3-butanediol in diets containing various levels of protein, fat, and carbohydrate. Metabolic and enzymatic studies are in progress.

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